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THE JAPANESE JOURNAL OF CANCER RESEARCH

Founded by K. YAMAGIWA and Continued by M. NAGAYO

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[GANN, Vol. 43, April, 1952]

ON THE CHROMOSOMES OF YOSHIDA SARCOMA. STUDIES WITH TUMOR CELLS PROLIFERATED IN THE PERITONEAL CAVITY OF THE RAT TRANSPLANTED WITH A SINGLE CELL.

(With Plates I—VIII)

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(Director: Prof. T. Yoshida)

INTRODUCTION

With various sorts of malignant tumor of men and animals painstaking researches have been made on chromosomes of tumor cells. But no phenomenon peculiar to malignant tumor has yet been noted. Haploidy, subdiploidy, diploidy, or polyploidy and many other variations of number of chromosomes are reported by many of researchers. I have preliminarily reported[1)2)] a result of my own investigation on the chromosome number of Yoshida sarcoma cells. Makino and others of Hokkaido University[3)4)] also gave their results with the same cells. Our conclusions were coincident with each other in that the number of chromosomes of the tumor cells was not fixed.

In view of the fact that the number of chromosomes of Yoshida sarcoma cells is not fixed, there arises the question: whether the tumor is a population of various strains of cells which have respective fixed numbers large or small, of chromosomes, or the number of chromosomes of the tumor cells is generally changeable according to varying conditions.

In the case of Yoshida sarcoma, as is with other transplantable tumors, ordinarily a large number of tumor cells are transplanted. Usually we inject into the peritoneal cavity of the rat 0.05–0.1 cc tumor ascites which contains 20–100 millions of cells in 0.1 cc.[5)] The transplantation with such large number of cells provides a confused basis for the answer to the above mentioned question.

Yoshida sarcoma has such a particular property as the tumor cell proliferates separated from another suspended in the peritoneal fluid. By taking advantage of this favourable character we can transplant with a single cell which has been picked out with the aid of a micromanipulator.[6)] And it has been an ascertained

fact that completely cell free ascites of Yoshida sarcoma[7][8] or broken-up cells[9] of the tumor have no longer transplantability. Therefore, in the case of single cell transplantation, the tumor cells found in the peritoneal cavity of the transplanted animals are undoubtedly those proliferated from the one cell transplanted.

In the present study on the chromosomes of Yoshida sarcoma cells, I employed exclusively those cells proliferated from a single cell in the above mentioned meaning.

Material and Methods

1. Into rats of about 100 gr. of mixed breed, one cell of Yoshida sarcoma was inoculated after Ishibashi and Hosokawa's method.[6][7] Among many such animals six were employed in the present work. In the case of one-cell-transplantation, compared with cases of usual transplantation with millions of cells, it needs rather longer period of time, chiefly in its initial growth. I classified the development of the tumor in the one cell cases into three stages.

1st stage: About 10 at 12 days after the transplantation a very few tumor cells are detected in the smear preparation of the ascites. After this the tumor cells increase in number relatively rapidly. In this stage one can find among widely scattered tumor cells in the smear preparations those which present excellent mitotic figures.

2nd stage: On about 13th to 16th day the tumor cells in the peritoneal cavity arrive at a state of pure culture of them. In the preparation one finds almost exclusively tumor cells, densely populated, and showing abundant mitotic figures.

3rd stage: About 23–26 days after the transplantation the animals die of malignant invasion of tumor cells. There are many degenerated cells, but not very much decreased in number of mitotic figures.

2. Preparations: In the research on chromosomes it has been an arduous labor to obtain adequate sections. Yoshida sarcoma has in this regard a great advantage, the cells being able to be observed by means of smear preparations. For the routine work with this tumor the Giemsa-stained preparations serve good enough, but for the purpose of the exact chromosome studies they are not sufficient. After comparative examinations of various methodes, I achieved a satisfactory effect by the following way, where the chromosomes were displayed in clear figures.

Here the smear preparation was fixed with corrosive-sublimate acetic alcohol without being dried, and stained with Heidenhein's iron haematoxylin.

(1) **Fixation:** Corrosive-sublimate acetic alcohol

Saturated mercuric chloride solution	100 cc (A)
96% alcohol	200 cc (B)

To the mixture of (A) and (B) glacial acetic acid is added in the proportion of 1 : 10 or 1 : 5. This fixation liquid is usable for one to two weeks. Tumor ascites is smeared on an object glass and immediately immersed in the fixation liquid with smeared surface downward, then kept in this condition in an incubator of 60° C for 30 minutes. Next it comes into 50% alcohol for 5 minutes, then is washed in 70% iodine alcohol to remove mercuric chrolide. After 10 to 15 minutes' washing it is kept in 96% alcohol for more than an hour.

(2) **Staining.** The preparation is placed successively in 70%, 50%, and 30% alcohol, then in distilled water. For 12 to 24 hours in 4% iron alum solution, a mordant. 5 to 10 minutes' washing in tap water. Then staining is carried on in 0.5% aqueous solution of haematoxylin for the same duration of time as in the mordant.

(3) **Differentiation.** For differentiation the preparation is placed in fresh 2% aqueous solution of iron alum till individual chromosomes appear clearly under the microscope; washed in running tap water for 20 to 30 minutes; then dehydrated in graded alcohol. Xylene is used for clarification. Mounting with balsam.

3. Drawing of the nuclear plates:

Among these completed preparations are picked out those showing satisfactorily stained figures in their metaphase of mitosis. They are drawn minutely with the aid of camera lucida (for examples Fig. 1–45 in Plates 1–4). Then the chromosomes of each drawn figure are arranged one by one in order of size (for examples Fig. 46–60 in Plates 5–6) and their numbers and forms examined.

Results and Considerations.

In one (No. 1) among 6 animals the chromosome number of the tumor cells was

Table 1. Chromosome number of tumor cells observed in each animal in each developing stage.

Animal	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
1st stage	34, 36, 37	42, 42, 44, 45 45, 45, 45, 45 49, 50, 54				
2nd stage	34, 38, 38, 42 43, 43, 44, 44 46, 46, 46, 47	45, 46, 42, 46	43, 46, 48	34, 42, 42, 42 43, 44, 47, 47 50, 91	42, 43, 45 46, 48, 49 49	
3rd stage	34, 42, 43, 32 36, 38, 39, 41 42, 42, 49, 33 33, 41		36, 38, 40 40, 32, 33 35, 36, 36 36, 37, 37 39. 40, 57		29, 35, 38	40, 41, 41 43, 44, 45 45, 46, 46 46, 47, 48 49, 49, 50 56, 73, 102

examined according to every stage of tumor developement (Tab. 1). There it is clearly demonstrated that the chromosome number of cells stemed from a single cell transplanted, fluctuates with a rather extended width in every developing stage of the tumor. This is true to all other 5 cases, each of which has been transplanted in the same way with a single cell, even if in these cases not all stages underwent the examination (Tab. 1).

If one, on Table 1, look at the case of No. 1 only, it seems as if the number of chromosomes is generally smaller than 42, the regular number in the germ cell of the rat, in earlier stages of tumor development. But in No. 2 all 11 cells out of the 1st stage present a larger number than 42. Therefore, in the evaluation of the results obtained, it is better, I think, that the results from 6 animals are all together taken into consideration.

1. Number of chromosomes.

a) Number and its frequency.

The number of chromosomes drawn on 100 nuclear plates are, as shown in Table 2, various, in an extensive range between 29 and 102. Table 3 is the

Table 2

Cell No.	Animal No.	Day after transplantation	Chromosome number	V-formed chromosome	Figure No.
1	No. 5	22nd	29	0	Pl. 1, Fig. 1
2	No. 3	20th	32	0	
3	No. 1	22th	32	0	
4	No. 3	20th	33	0	Pl. 1, Fig. 2
5	No. 1	25th	33	0	
6	No. 1	25th	33	0	
7	No. 1	11th	34	0	Pl. 1, Fig. 3
8	No. 4	14th	34	smaller 1	Pl. 1, Fig. 4
9	No. 1	14th	34	larger 1	Pl. 3, Fig. 24
10	No. 1	19th	34	0	
11	No. 5	22nd	35	0	Pl. 1, Fig. 5
12	No. 3	20th	35	0	
13	No. 1	11th	36	0	Pl. 1, Fig. 6
14	No. 3	18th	36	0	
15	No. 3	20th	36	0	Pl. 1, Fig. 7
16	No. 3	20th	36	0	
17	No. 3	20th	36	0	

Table 2—2.

Cell No.	Animal No.	Day after transplantation	Chromosome number	V-formed chromosome	Figure No.
18	No. 1	22nd	36	larger 1	Pl. 3, Fig. 25
19	No. 1	11th	37	0	Pl. 1, Fig. 8
20	No. 3	20th	37	smaller 1	Pl. 3, Fig. 26
21	No. 3	20th	37	0	
22	No. 1	14th	38	0	
23	No. 1	14th	38	0	Pl. 1, Fig. 9
24	No. 3	18th	38	0	
25	No. 1	22th	38	0	
26	No. 5	22nd	38	larger 1 & smaller 1	Pl. 3, Fig. 27
27	No. 3	20th	39	0	
28	No. 1	22nd	39	0	Pl. 1, Fig. 10
29	No. 3	18th	40	0	
30	No. 3	18th	40	0	
31	No. 3	20th	40	0	
32	No. 6	22nd	40	larger 1	Pl. 3, Fig. 28
33	No. 1	22nd	41	larger 1	
34	No. 6	22nd	41	0	Pl. 1, Fig. 12
35	No. 6	22nd	41	larger 1	Pl. 3, Fig. 29
36	No. 1	25th	41	0	Pl. 1, Fig. 11
37	No. 2	12th	42	smaller 1	
38	No. 2	12th	42	smaller 2	Pl. 3, Fig. 31
39	No. 4	14th	42	smaller 4	Pl. 3, Fig. 30
40	No. 4	14th	42	larger 1	
41	No. 4	14th	42	0	
42	No. 1	14th	42	0	
43	No. 5	14th	42	smaller 1	
44	No. 2	16th	42	0	
45	No. 1	19th	42	0	Pl. 2, Fig. 14
46	No. 1	22nd	42	0	Pl. 2, Fig. 13
47	No. 1	22nd	42	0	
48	No. 3	14th	43	larger 1	Pl. 3, Fig. 32
49	No. 4	14th	43	0	
50	No. 1	14th	43	0	Pl. 2, Fig. 15
51	No. 1	14th	43	0	
52	No. 5	14th	43	smaller 1	

Table 2—3.

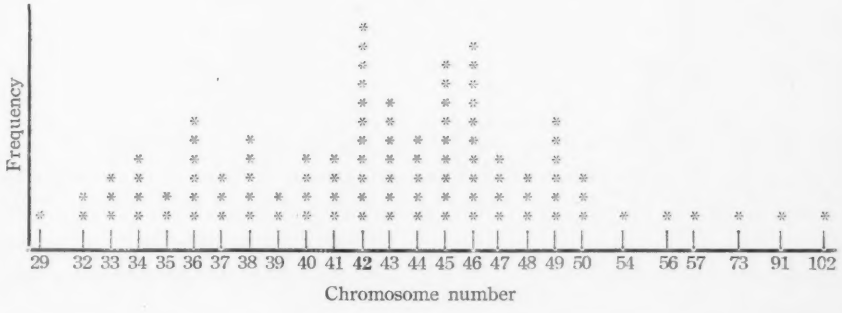
Cell No.	Animal No.	Day after transplantation	Chromosome number	V-formed chromosome	Figure No.
53	No. 1	19th	43	larger 1	
54	No. 6	22nd	43	larger 1	Pl. 3, Fig. 33
55	No. 2	12th	44	smaller 1	
56	No. 4	14th	44	smaller 3	
57	No. 1	14th	44	0	
58	No. 1	14th	44	larger 1	Pl. 3, Fig. 34
59	No. 6	22nd	44	0	
60	No. 2	12th	45	smaller 2	
61	No. 2	12th	45	smaller 2	
62	No. 2	12th	45	smaller 1	
63	No. 2	12th	45	larger 1 & smaller 1	Pl. 3, Fig. 35
64	No. 2	12th	45	smaller 1	
65	No. 2	14th	45	0	
66	No. 5	14th	45	smaller 2	
67	No. 6	22nd	45	0	
68	No. 6	22nd	45	larger 1	Pl. 4, Fig. 36
69	No. 3	14th	46	smaller 2	
70	No. 2	14th	46	0	Pl. 2, Fig. 17
71	No. 1	14th	46	larger 1	Pl. 4, Fig. 37
72	No. 1	14th	46	smaller 3	
73	No. 1	14th	46	0	
74	No. 5	14th	46	0	
75	No. 2	16th	46	larger 1 & smaller 2	
76	No. 6	22nd	46	0	
77	No. 6	22nd	46	0	Pl. 2, Fig. 16
78	No. 6	22th	46	0	
79	No. 4	14th	47	smaller 3	Pl. 4, Fig. 38
80	No. 4	14th	47	0	
81	No. 1	14th	47	0	Pl. 2, Fig. 18
82	No. 6	22nd	47	smaller 1	Pl. 4, Fig. 39
83	No. 3	14th	48	larger 1	
84	No. 5	14th	48	larger 1 & smaller 2	Pl. 4, Fig. 40
85	No. 6	22nd	48	0	Pl. 2, Fig. 19
86	No. 2	12th	49	0	Pl. 2, Fig. 20

Table 2—4.

Cell No.	Animal No.	Day after transplantation	Chromosome number	V-formed chromosome	Figure No.
87	No. 5	14th	49	smaller 2	
88	No. 5	14th	49	0	Pl. 2, Fig. 21
89	No. 1	22nd	49	larger 1	
90	No. 6	22nd	49	larger 1	
91	No. 6	22nd	49	0	Pl. 2, Fig. 22
92	No. 2	12th	50	smaller 1	Pl. 4, Fig. 41
93	No. 4	14th	50	0	
94	No. 6	22nd	50	0	Pl. 2, Fig. 23
95	No. 2	12th	54	smaller 1	Pl. 4, Fig. 42
96	No. 6	22nd	56	larger 1	Pl. 4, Fig. 43
97	No. 3	20th	57	larger 1	
98	No. 6	22nd	73	0	Pl. 3, Fig. 44
99	No. 4	14th	91	0	
100	No. 6	22nd	102	0	Pl. 4, Fig. 45

graphic exhibition of the variations of chromosome number. 11 plates have 42 chromosomes, 10 have 46, 9 have 45, 7 have 43. Those having 36 and 49 chromosomes are 6 each. 38 and 44 of chromosomes are found on 5 plates each. Those

Table 3. Graphic exhibition of the variation of chromosome number of Yoshida sarcoma cells.



having more than 50 and less than 30 are only 7 in all. After all, the most frequent numbers of chromosomes are between 42 and 46, amounting to 42 cases out of 100.

b) Relation between chromosome number and tumor development.

Table 4 is a classification of the 100 nuclear plates by chromosome number and

Table 4. Chromosome number and periodical frequency.

	-35	36-40	41-45	46-50	51-	Total
1st stage	1	2	8	2	1	14
2nd stage	2	2	16	15	1	36
3rd stage	9	16	12	9	4	50
Total	12	20	36	26	6	100

period after transplantation. In the 1st stage of tumor development the numbers from 41 to 45 are prevailing, in the 2nd stage the same numbers are also most frequently seen followed by those of 46 to 50. But in the 3rd stage the number in the column 41 to 45 is surpassed by that in the column 36 to 40. On the whole, chromosome numbers between 41 and 45 make the majority of 100 nuclear plates examined, amounting to 36% of them. The numbers from 46 to 50, from 36 to 40 follow this. It must be noticed that 82% of the whole nuclear plates examined, fall in the columns of 36 to 50.

In Table 5 the numbers of chromosomes are divided into three divisions, 42, more

Table 5. Variations of the chromosome numbers of the tumor cells from the regular number of the rat cell chromosomes 42.

	below 42	42	above 42
1st stage	3	2	9
2nd stage	4	6	26
3rd stage	29	3	18
Total	36	11	53

than 42 and less than 42, and their frequency is given in each stage of tumor development. The number 42 is said to signify the regular number of chromosomes in a spermatid of a normal rat.[10] In the 1st and 2nd stages there are seen with greater frequency cells whose number of chromosomes is more than the regular number, while in the 3rd stage those with less than 42 chromosomes are greater in number.

From the above, the following conclusions may be reached.

(1) The chromosome numbers of tumor cells proliferated from a single cell are subject to wide variations.

(2) The chromosome numbers are in most cases in the neighbourhood of the

regular number 42, but not fixed.

(3) In the very early stage of tumor growth it is already clearly known that the chromosome numbers are not fixed. In the early stage the number of cells living in the peritoneal cavity is small, consequently their living conditions are not so abnormal ordinarily. If there is any fixed number in the chromosomes of tumor cells, the number is expected for almost all of the cells in this stage. Because the fact is to the contrary, it is unthinkable that tumor cells have a fixed number of chromosomes, as normal spermatids have the fixed number 42.

(4) It is worthy of note that in the state of beginning pure culture of tumor cells in the peritoneal cavity, cells whose chromosome number is over 42 are considerably many, while in the later stage where many cells tend to degenerate, those with fewer than 42 chromosomes are observed in greater number.

2. Form of chromosomes.

As shown in Figures 46–60 in Plates 5–6, the form and size of individual chromosomes are so variable in each nuclear plate that the arrangement in pairs of homologous chromosomes is in major cases, without arbitrary choice, impossible. So I arranged them simply according to the decreasing size.

a) V-shaped chromosomes.

A characteristic fact to be marked concerning the form of chromosomes is the existence of V-shaped ones (see Fig. 4, Figg. 24–43). 42 chromosomes of a spermatid of a rat are all of rodshape. Of V-shaped chromosomes we have no record in rat cells. In the polar view of mitotic figures in their metaphase chromosomes are seen arranging themselves almost radially. Among which those that have a shape of V, with their points towards the center of the equatorial plate we call V-shaped ones.

I found in 42 out of 100 nuclear plates examined the V-shaped chromosomes. Among these 42, 28 plates contain only one of this shape, while in the remaining

Table 6. Number of V-shaped chromosomes and their frequency in each stage of tumor growth.

Number	1	2	3	4
1st stage	6	4	0	0
2nd stage	9	3	5	1
3rd stage	13	1	0	0
Total	28	8	5	1

14 plates the number of V-shaped chromosome varies from 2 to 4 (Tab. 6).

V-shaped chromosomes are of different sizes, so that in the arrangement of chromosomes of each cell in decreasing order of size, they are placed in every point in the arrangement, wandering from the top almost to the end (Plates 5–6). Therefore, when they are described simply large or small, it may sound confused. I tried to classify them broadly into two groups, larger and smaller, the former belonging to the half of larger sized chromosomes in the arrangement, the later to the rest.

(b) **Frequency of appearance of the nuclear plate with V-shaped chromosome.** Out of 100 nuclear plates, 42 have V-shaped chromosomes, larger or smaller; 58 containing none of them at all. Periodically speaking, in earlier stage cells containing V-shaped chromosomes are larger in number than those which are

Table 7. Relation of the appearance of V-shaped chromosomes to the total number of chromosomes of the tumor cells.

Chromosome number	-35		36-40		41-45		46-50		51-		Total	
V-shaped chromosome	+	−	+	−	+	−	+	−	+	−	+	−
1st stage	0	1	0	2	8	0	1	1	1	0	10	4
2nd stage	2	0	0	2	8	8	8	7	0	1	18	18
3rd stage	0	9	4	12	5	7	3	6	2	2	14	36
Total	2	10	4	16	21	15	12	14	3	3	42	58

devoid of such ones, and in the later stages this relation becomes reversed (Tab. 7).

As for the total chromosome number of the nuclear plates containing V-shaped chromosomes, those having 41 to 50 chromosomes include in more cases V-shaped ones, while in those with less than 41 of chromosomes the reverse is the case (Tab. 7).

Of the nuclear plates having the regular number or more of chromosomes,

Table 8. Variations of the chromosome numbers of the nuclear plates containing V-shaped ones from the regular chromosome number 42.

Chromosome number	below 42		42		above 42	
V-shaped chromosome	+	−	+	−	+	−
1st stage	0	3	2	0	8	1
2nd stage	2	2	3	3	13	13
3rd stage	6	23	0	3	8	10
Total	8	28	5	6	29	24

those including V-shaped chromosome are in total a little more than those without them. But in plates with less than 42 chromosomes there are far more of those without any V-shaped chromosomes (Tab. 8).

When frequency is looked up as of groups of larger or smaller size, V-shaped chromosomes of larger size tend to appear in the later stages, while those of smaller size show the tendency to appear in greater number in earlier stages. Out of 42 nuclear plates with V-shaped chromosomes, 17 have larger one, 21 smaller one and the remaining 4 have both larger and smaller V-shaped chromosomes (Tab. 9).

Table 9. Frequency of larger and smaller V-shaped chromosomes.

	Number of observed nuclear plate	larger V.		smaller V.	
		frequency	%	frequency	%
1st stage	14	1	7.1	10	71.4
2nd stage	36	8	22.2	12	33.3
3rd stage	50	12	24.0	3	6.0
Total	100	21	21.0	25	25.0

The above description as to the form of chromosomes may be summarized as follows:

(1) The greater number of chromosomes are of rod-shape or grain-form.

(2) Out of 100 nuclear plates 42 have V-shaped chromosomes.

(3) V-shaped chromosomes are to be observed in every period of the growth of tumor.

(4) The existence of V-shaped chromosomes has nothing to do with the fixed number of chromosomes.

As for the significance of V-shaped chromosomes Makino has proposed a opinion based on his own observations.[11] But from the result of my present studies it is, I think, not yet safe to attribute V-shaped chromosomes a special meaning concerning the genesis of cancer cells. Cells with V-chromosomes do not seem to make a special stem of cell, for among 100 cells examined those having V-shaped chromosomes are less than the half of the total cells (42%), and the remaining cells have none of such ones. Further, the percentage among the cell groups proliferated from a single cell, varies in six groups studied as follows: 27.7, 31.0, 38.8, 50, 50, 73.3 percent (Tab. 10). This inconstancy in the frequency of V-shaped chromosomes in cells proliferated from a single cell is very likely to indicate that the V-shaped chromosomes can appear in any cell of this tumor depending on some yet unknown condition of cell life. It may be noticed further

that the number of chromosomes of nuclear plates which contains V-shaped ones are subject to extensive variations.

Table 10. Frequency of V-shaped chromsomes among six animals transplanted with one cell

Animal	V-shaped chromosome		(C) Total	A/C%
	(A) +	(B) −		
No. 1	9	20	29	31.0
No. 2	11	4	15	73.3
No. 3	5	13	18	27.7
No. 4	5	5	10	50.0
No. 5	5	5	10	50.0
No. 6	7	11	18	38.8

Conclusion

A single cell of the Yoshida sarcoma was transplanted into the peritoneal cavity of rat, then the chromosomes of mitotic cells issued from the cell were observed.

1) The number of chromosomes vary widely between 35 and 50, the regular number of the spermatid of rats, 42, being the center. In other words, the transplantation with a single cell dose not fix the chromosome number.

2) V-shaped chromosomes, not seen in case of normal rats, were noticed on 42 out of 100 nuclear plates, but any special stem of cells containing V-shaped chromosomes is not decided, such chromosomes appearing on plates with various numbers of chromosomes.

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Explanation of Plates I & II.

Drawn figures of chromosomes of Yoshida sarcoma cells with the aid of camera lucida. Except in Fig. 4, any V-shaped chromosome is not recognized. Magnification, ca. ×3000.

Figure 1. Chromosome number 29, observed in the preparation of the 22nd day tumor ascites after intraperitoneal transplantation with a sigle cell of Yoshida sarcoma.
Figure 2. Chromosome number 33, in the 10th day, ascites.
Figure 3. 〃 34, in the 11th day, ascites.
Figure 4. 〃 34, in the 14th day, ascites.
Figure 5. 〃 35, in the 22nd day, ascites.
Figure 6. 〃 36, in the 11th day, ascites.
Figure 7. 〃 36, in the 20th day, ascites.
Figure 8. 〃 37, in the 11th day, ascites.
Figure 9. 〃 38, in the 14th day, ascites.
Figure 10. 〃 39, in the 22nd day, ascites.
Figure 11. 〃 41, in the 25th day, ascites.
Figure 12. 〃 41, in the 22nd day, ascites.
Figure 13. 〃 42, in the 22nd day, ascites.
Figure 14. 〃 42, in the 19th day, ascites.
Figure 15. 〃 43, in the 14th day, ascites.
Figure 16. 〃 46, in the 22nd day, ascites.
Figure 17. 〃 46, in the 14th day, ascites.
Figure 18. 〃 47, in the 14th day, ascites.
Figure 19. 〃 48, in the 22nd day, ascites.
Figure 20. 〃 49, in the 12th day, ascites.
Figure 21. 〃 49, in the 14th day, ascites.
Figure 22. 〃 49, in the 22nd day, ascites.
Figure 23. 〃 50, in the 22nd day, ascites.

Explanation of Plates III & IV.

Drawn figures of the nuclear plates with the V-shaped chromosomes, except in Figg. 44, 45 Magnification, ca. ×3000.

Figure 24. Chromosome number 34, observed in the 14th day ascites, larger 1 of V-shaped chromosome.
Figure 26. Cnromosome number 36, 22nd day, larger. 1.
Figure 26. 〃 37, 20th day, smaller 1.
Figure 27. 〃 38, 22nd day, larger 1 & smaller 1.

Figure 28. 〃 40, 22nd day, larger 1.
Figure 29. 〃 41, 22nd day, larger 1.
Figure 30. 〃 42, 14th day, smaller 4.
Figure 31. 〃 42, 12th day, smaller 2.
Figure 32. 〃 43, 14th day, larger 1.
Figure 33. 〃 43, 22nd day, larger 1.
Figure 34. 〃 44, 14th day, larger 1.
Figure 35. 〃 45, 12th day, larger 1 & smaller 1.
Figure 36. 〃 45, 22nd day, larger 1.
Figure 37. 〃 46, 14th day, larger 1.
Figure 38. 〃 47, 14th day, smaller 3.
Figure 39. 〃 47, 22nd day, smaller 1.
Figure 40. 〃 48, 14th day, larger 1 & smaller 2.
Figure 41. 〃 50, 12th day, smaller 1.
Figure 42. 〃 54, 12th day, smaller 1.
Figure 43. 〃 56, 22nd day, larger 1.
Figure 44. 〃 73, 22nd day, no V element.
Figure 45. 〃 102, 22nd day, no V element.

Explanation of Plates V & VI.

Arrangements of chromosomes in order of size. Except in Figg. 46, 47, 48, 51 & 53, are found 1 to 4, larger and/or smaller V-shaped chromosomes.

Figure 46. Arrangement of chromosomes of Fig. 3, number 34.
Figure 47. Arrangement of chromosomes of Fig. 5, number 35.
Figure 48. Arrangement of chromosomes of Fig. 6, number 36.
Figure 49. Arrangement of chromosomes of Fig. 27, number 38, larger 1 and smaller 1 of V-shape.
Figure 50. Arrangement of Fig. 28, number 40, larger 1.
Figure 51. 〃 of Fig. 12, number 41,
Figure 52. 〃 of Fig. 29, number 41, larger 1.
Figure 53. 〃 of Fig. 13, number 42,
Figure 54. 〃 of Fig. 31, number 42, smaller 2.
Figure 55. 〃 of Fig. 30, number 42, smaller 4.
Figure 56. 〃 of Fig. 33, number 43, larger 1 & smaller 1.
Figure 57. 〃 of Fig. 32, number 43, larger 1.
Figure 58. 〃 of Fig. 34, number 44, larger 1.
Figure 59. 〃 of Fig. 35, number 45, larger 1 & smaller 1.
Figure 60. 〃 of Fig. 41, number 50, smaller 1.

Explanation of Plates VII & VIII.

Microphotographs of the mitotic cells observed from the smear preparations of Yoshida sarcoma, stained with Heidenhein's ironhaematoxilin. Photographed using Obj. ×90, Occ. ×10 (Zeiss), and "Phoke' (Zeiss). Magnification, ca. ×2100-2400.

Figure 61. Microphotograph of the cell of Fig. 1.
Figure 62. 〃 〃 Fig. 3.
Figure 63. 〃 〃 Fig. 5.
Figure 64. 〃 〃 Fig. 6.

Figure 65.	〃	〃	Fig. 7.
Figure 66.	〃	〃	Fig. 8.
Figure 67.	〃	〃	Fig. 10.
Figure 68.	〃	〃	Fig. 12.
Figure 69.	〃	〃	Fig. 13.
Figure 70.	〃	〃	Fig. 14.
Figure 71.	〃	〃	Fig. 22.
Figure 72.	〃	〃	Fig. 23.
Figure 73.	〃	〃	Fig. 24.
Figure 74.	〃	〃	Fig. 25.
Figure 75.	〃	〃	Fig. 27.
Figure 76.	〃	〃	Fig. 28.
Figure 77.	〃	〃	Fig. 29.
Figure 78.	〃	〃	Fig. 31.
Figure 79.	Microphotograph of the cell of		Fig. 33.
Figure 80.	〃	〃	Fig. 36.
Figure 81.	〃	〃	Fig. 39.
Figure 82.	〃	〃	Fig. 43.
Figure 83.	〃	〃	Fig. 44.
Figure 84.	〃	〃	Fig. 45.

要　旨

吉田肉腫の染色体の研究（一個の細胞から増殖した腫瘍細胞についての観察）

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（東北大学病理学教室，指導吉田富三教授）

一個の吉田肉腫細胞を白ねずみの腹腔内に移植し，それから増殖した腫瘍細胞の染色体を描画して，数及び形の研究を行つた。

1) 数には最小 29 から最多 102 迄，非常に幅の廣い変動がみられた。大部分は白ねずみ精細胞の染色体の常数である 42 を中心として，35 から 50 迄の間の数を示している。しかし数は移植後のどの時期にも一定しない。即ち1個からしふやてみても，その子孫の細胞の染色体数に変動が起る。

2) 染色体の形について最も大きた事実は，V 型の染色体の出現である。白ねずみ精細胞の染色体は全部棒状で，このような形のものは記載されていない。従ってこれは腫瘍細胞に特長的なものである。V 型は 100 個の中 42 の核板にみられた。V型には染色体を大きさの順に配列した場合に順列の中央以上に並ぶ大型と，以下に並ぶ小型のものとがある。大小の V 型を併せ持つものも少数あるが，大部分は何れか 1 ケのものが多い。又 V 型は腫瘍成長のどの時期にもみられる。染色体数 35 以下又は 50 以上というようなものには，V 型の出現は少いが，35 から 50 迄の染色体数のものには，V 型をもつものともたないものがどの数の所にもあり，特に染色体数いくつのものに V 型があるという特定の関係はみとめられない。

一個の細胞からふやした子孫の細胞にも V 型をもつものともたぬものがあり，且つその出現は約 40% であるという結果から，V 型を有する特定の細胞系統を設定して，V 型を有しないものを変性或は変則的なものと判断することは困難である。（文部省科学研究費による）

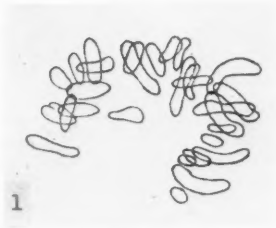


Fig. 1

Fig. 2

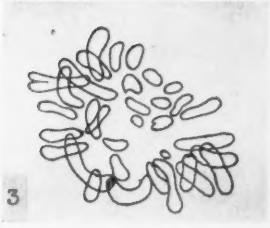


Fig. 3

Fig. 4

Fig. 5

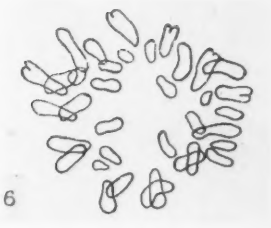


Fig. 6

Fig. 7

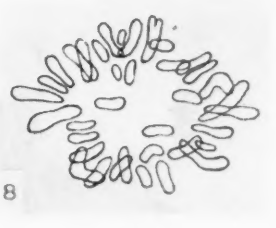


Fig. 8

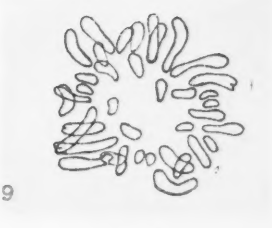


Fig. 9

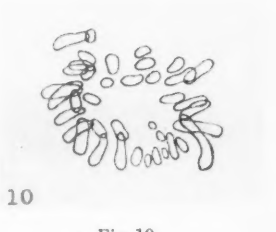


Fig. 10

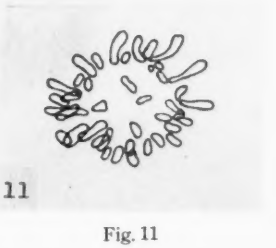
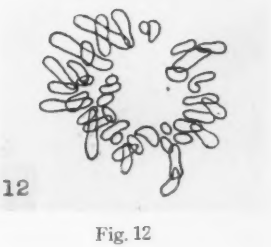


Fig. 11



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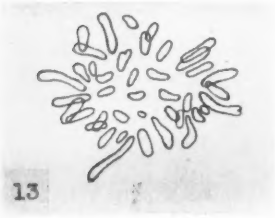
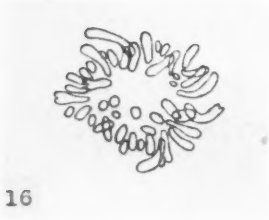


Fig. 13

Fig. 14

Fig. 15



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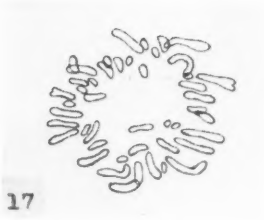

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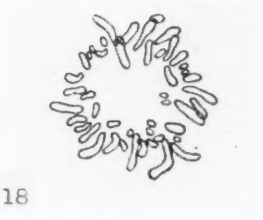

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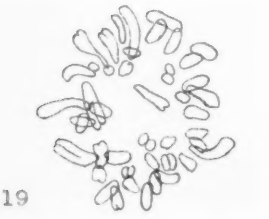

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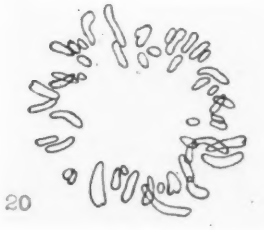


Fig. 20

Fig. 21

Fig. 22

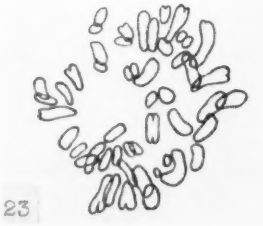


Fig. 23

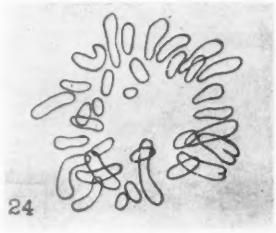


Fig. 24

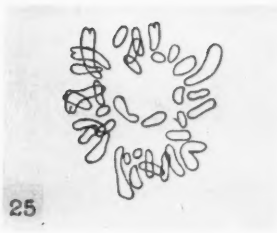


Fig. 25

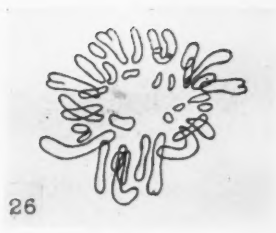
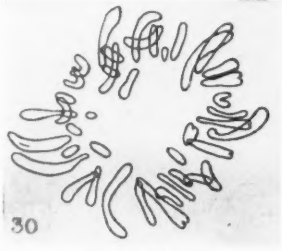


Fig. 26

Fig. 27

Fig. 28

Fig. 29



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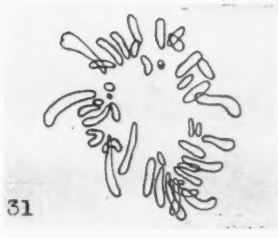

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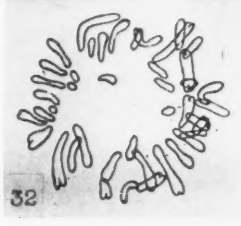

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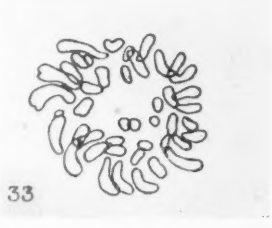

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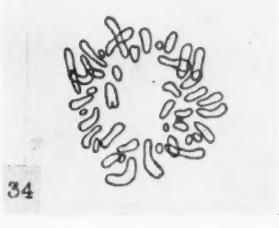

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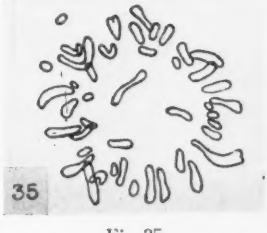

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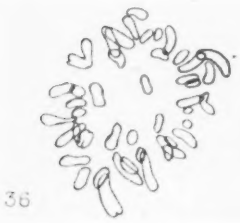

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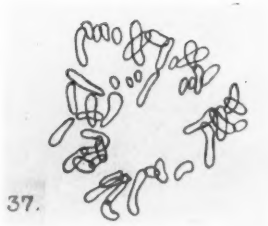

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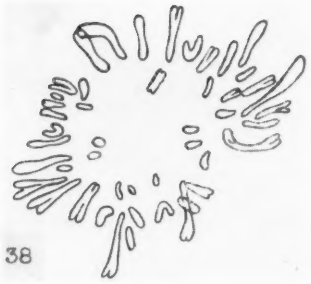

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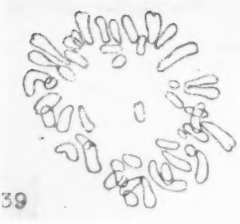


Fig. 39

Fig. 40

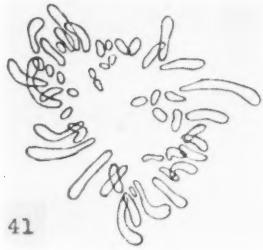


Fig. 41

Fig. 42

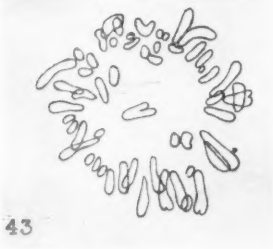


Fig. 43

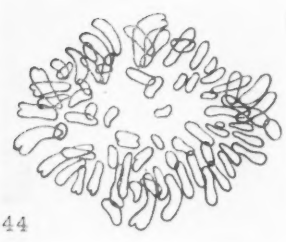


Fig. 44

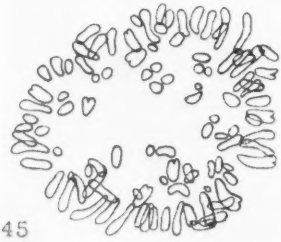


Fig. 45

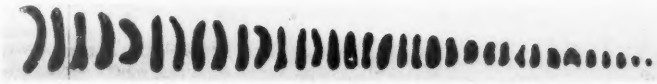


Fig. 46

Fig. 47

Fig. 48

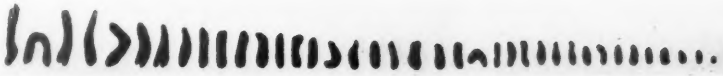


Fig. 49

Fig. 50

Fig. 51

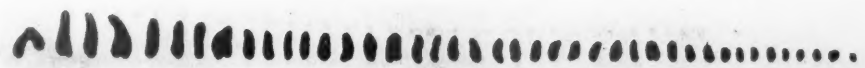


Fig. 52

Fig. 53

Fig. 54

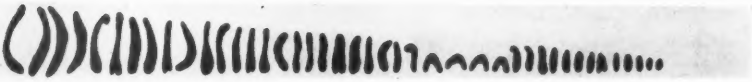


Fig. 55

Fig 56

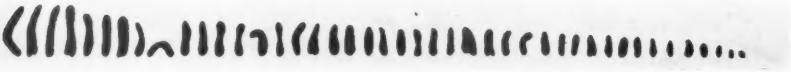


Fig. 57

Fig. 58

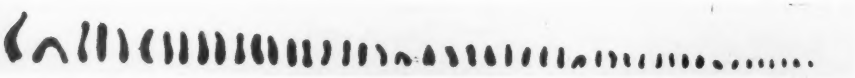


Fig. 59

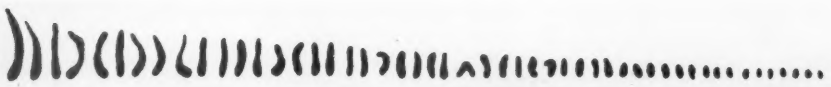


Fig. 60

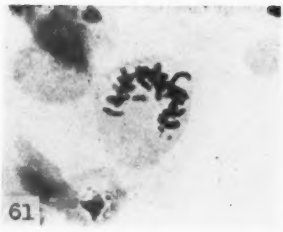


Fig. 61

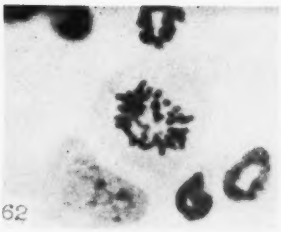


Fig 62

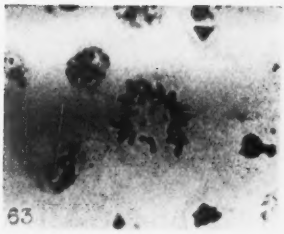


Fig. 63

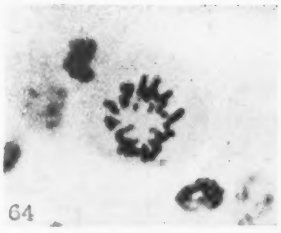


Fig 64

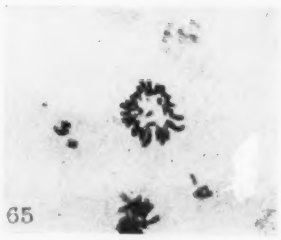


Fig 65

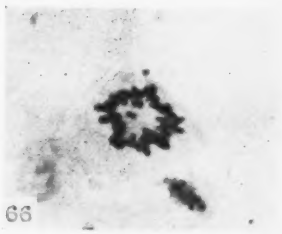


Fig. 66

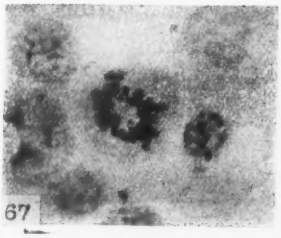


Fig. 67

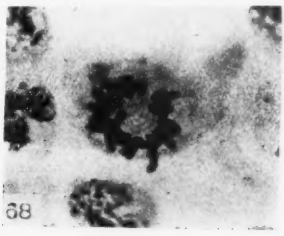


Fig. 68

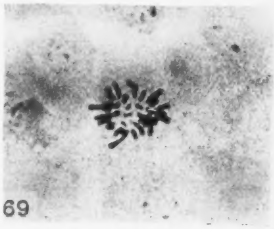


Fig. 69

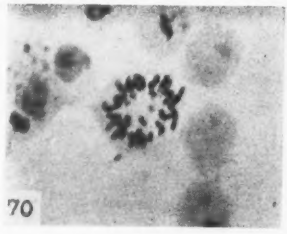


Fig. 70

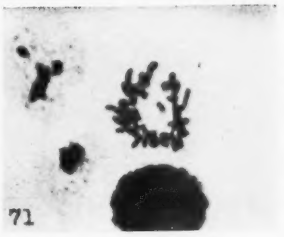


Fig. 71

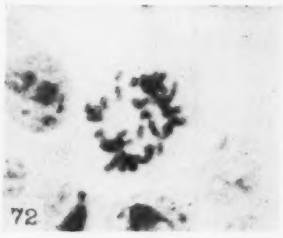


Fig. 72

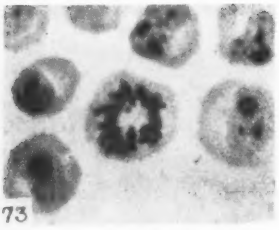


Fig. 73

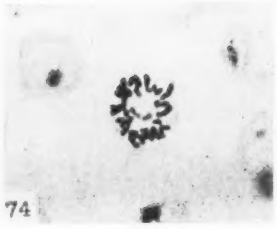


Fig. 74

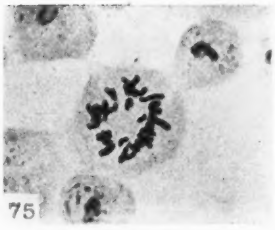
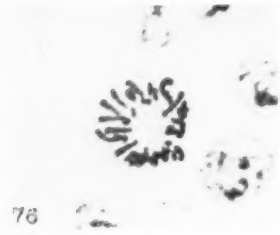


Fig. 75



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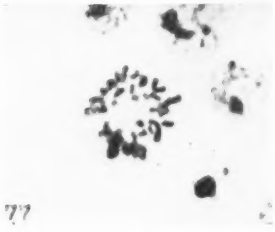

Fig. 77

Fig. 78

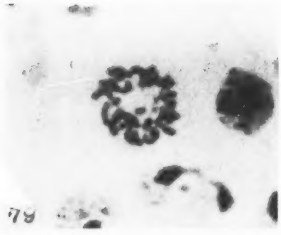

Fig. 79

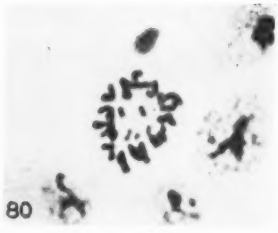

Fig. 80

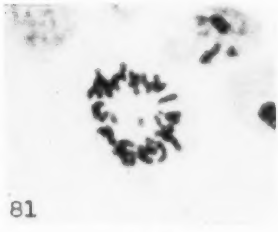

Fig. 81

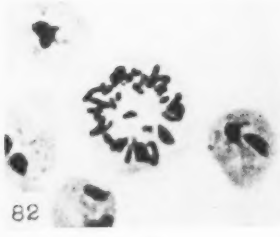

Fig. 82

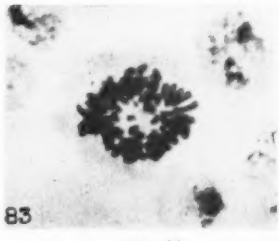

Fig. 83

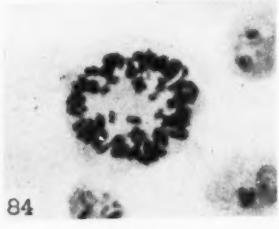

Fig. 84

[GANN, Vol. 43, April, 1952]

CYTOLOGICAL STUDIES ON CANCER. III. THE CHARACTERISTICS AND INDIVIDUALITY OF CHROMOSOMES IN TUMOR CELLS OF THE YOSHIDA SARCOMA WHICH CONTRIBUTE TO THE GROWTH OF THE TUMOR

(With Plates IX—XI)

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From the results of daily observations of the mitotic rate and the variation of the chromosome number in tumor cells of the Yoshida sarcoma through a transplant generation, it was concluded in the former studies (Makino and Yosida 1951, Makino and Kanô 1951) that there is a strain of tumor cells which primarily contribute to the formation of the tumor; they are characterized by well-balanced subdiploid chromosomes, 40 or thereabouts in number and almost ordinary in their metaphase configuration. The situation further demands an investigation of the chromosome morphology of these tumor cells, going into the subject more deeply than in the former studies, particularly inquiring into the morphological analysis of chromosomes. Furthermore, interest has also arisen as to the question whether the chromosomes are alike or not in the tumor cells and in those of the host animal. Taking these things under consideration, investigation should be extended to the morphological analysis of chromosomes together with their behavior during mitosis in the tumor cells of the Yoshida sarcoma, in as great detail as possible and with some experiments. The results of investigation with such points in mind are herein presented.

The major part of the present observations were carried out with the acetocarmine smear preparations of tumor cells (for procedure refer to the former papers, Makino and Yosida 1951 a; M. and Kanô 1951 b). In the latter part of this study, Tanaka (1951), one of our co-workers, advised the use of acetic gentianviolet which gives results that are equally as good as those by the use of acetocarmine, so that some of the preparations were made after the acetic gentianviolet method with successful results.

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The author is happy to offer his sincere thanks to Emeritus Professors, Dr. Kan Oguma and Dr. Yoshinari Kuwada for their important criticism and keen interest. Gratitude must also be extended to Professor Tetsu Sakamura for his valuable advice, especially for the experiment of permeability. Further the author is also indebted to Miss Kyoko Kanô, Mr. Tatsuya Tanaka and Mr. Hiroshi Nakahara for their kind aids during the course of the study.

Observations

1. Morphological analysis of the chromosomes in tumor cells

It was pointed out from previously reported observations, which dealt with the daily surveys of the mitotic rate and chromosome number in tumor cells through a whole life span of the tumor rat, that the tumor cells with well-balanced subdiploid chromosomes, 40 or thereabouts in number, are significant in connection with the growth of the tumor on account of their striking high frequency and definite mitotic rate, and further that the variation of the chromosome number takes place around 40, showing a gradual fluctuation both above and below that number (Makino and Kanô 1951). Particularly, the cells with 38, 39, 40 and 41 chromosomes show a remarkably high rate of occurrence (cf. Table 5 in Makino and Kanô 1951). In this study the chromosomes were investigated in these tumor cells with special concern as to their morphological feature and by way of comparison with those of the host, *Rattus norvegicus*.[1]

Figures 1 to 6 are examples of the metaphase plates of tumor cells which show the chromosome numbers of 38, 40, 40, 41, 42 and 43, respectively. In every one of them, the chromosomes are arranged in an ordinary radial manner; the larger chromosomes generally occupy a peripheral position in the equatorial plate, with the smaller ones in the central space, each directing the points of fiber attachment towards the centre of the plate. Furthermore, there is nothing unusual in the orientation of chromosomes nor in the spindle formation. This renders the study of the morphology of individual chromosomes easy.

By a glance at the metaphase plates (Figs. 1–6, and Figs. 61–63), the chromosome complex of tumor cells is seen to be very motley because of the prevalence of J-and V-like elements of various shapes and sizes. Among them, the existence of a prominent large V-shaped element is specially notable. It is very surprising to find such a motley chromosome complex in tumor cells, since the chromosomes

1) It should be mentioned that the observations of chromosomes were made by co operation of Miss Kanô and Mr. Nakahara and myself based on material derived from some different rats, respectively. The results of these investigations independently carried out by three workers fell into a complete agreement

of the host animal (*Rattus norvegicus*) are 42 in number and all are rod-type in superficial appearance (cf. Figs. 25–28). These characteristic features facilitate the distiguishing of the individual chromosomes from each other. Based on the comparison of their characteristic shapes and sizes the individual chromosomes were morphologically analysed as accurately as possible, and were then placed into supposed, not real, homologous pairs ranging in serial order of size. Examples of the serial alignment are given in Figures 11 to 22. By reference to these figures, it is very apparent that, disregarding the variation in chromosome number, there is still a clear-cut distinction of the chromosomes into two remarkable sets, viz., the one consisting of rod-shaped elements and the other including those of V- and J-shaped ones. In all probability, the rod-shaped chromosomes seem to constitute 12 pairs, though there occur some cases in which certain chromosomes are unpaired, or in which some elements exist in excess, probably as the result of non-disjunction, lagging or such like irregularities. They show a gradual decrease in langth forming a graded seriation which rank from extremely elongated elements to small ones, excepting in the smallest ones remarkable in size. Frequently, some lerger chromosomes carry a remarkable globular part at the proximal end of the main body, which globular part shows a slight bending, thus displaying a J-shape. At present, it may not be said with certainty whether these chromosomes are of subterminal nature or not, since their inner essential structure remains unknown. Therefore, the author should like to consider them tentatively as rod-shaped chromosomes. Based on observations of a considerable number of the metaphase plate, it appears that, especially in the set of the rod chromosomes, the elements are paired into a natural or well-balanced state.

The chromosome set consisting of V- and J-shaped elements comprises, on the other hand, nine or occasionally ten supposed pairs. It is of course uncertain whether the elements placed into pairs are really homologous mates. The larger two or three pairs are apparent on account of the prominent size and distinct V- or J- shape, while the remaining chromosomes are uniformly small in size forming a nearly graded series. They all bear an apparent constriction at their middle part or near one extremity in each and at the point of constriction they exhibit a gentle bending. In the metaphase plates as seen in Figures 1–6, these elements usually dispose themselves with the point of constriction directed towards the centre of the equatorial plate. These pictures seem to favour the inference that they are not simple rod-type chromosomes but of submedian or subterminal nature, since the constriction probably corresponds to the point of the fibre attachment in each. In addition to these chromosomes, there is present a striking V-shaped element of large size, being in a completely solitary state without a mate. This chromosome seems to be of submedian fiber attachment because of

its dissimilar two arms; its long arm is seen to correspond in magnitude to the members of the 4th or 5th pair in the rod-type group. There is present no chromosomes corresponding to it in either size or shape in the complement. Therefore, the karyotype of tumor cells is notably marked by the occurrence of this V-element.

The V-shaped chromosomes are likewise clearly discernible either in hypoploid or polyploid cells. Figure 8 is an example of a hypoploid cell with a very reduced chromosome number, while Figures 9 and 10 exhibit subtriploid and subtetraploid cells, respectively. The same regarding V-shaped chromosomes is to be said as to the giant cell with a very high chromosome number, though in the giant cell the chromosomes are usually small in size and therefore the morphological demarkation of individual elements is much less distinct. Particularly, the presence of the remarkable V-element of large size is prominent in the giant cells. The subtriploid cells usually show two, but occassionally one, such V-element, while there are in most cases two V's in the subtetraploid cells.

Summing up,[1] it may be stated that the tumor cells which primarily play a significant role in the proliferation of the tumor have well-balanced subdiploid chromosomes, 40 or thereabouts in number; the chromosome constitution of these cells is very characteristic in comprising 22 to 24 elements of rod-type forming a graded series and 16 to 18 elements assuming J- and V-shape of various sizes, among them a large V-shaped element of submedian nature being outstanding.

2. The behavior of chromosomes in tumor cells during mitosis

Next, attention was paid to the mitotic behaviour of chromosomes in the strain cells of tumor which are remarkable, as described above, because of the appreciable characteristics of the chromosomes, being 40 or thereabouts in number, and having a motley constitution comprising various rod-, V- and J-elements. It was found that the behaviour of chromosomes in these cells during mitosis is regular showing no visible evidence of abnormality, so far as the observations have gone with the material taken from the middle period of the life of the host, when the proliferation of tumor cells is most active. The sequence of events is shown in the accompanying figures (Figs. 56–60).

The resting nuclei of tumor cells are oval or kidney-shaped, and usually contain one or two, sometimes three, nucleoli which are negative in Feulgen reaction. Figure 56 indicates the prophase, showing the regular course of development of chromosomes in the nucleus. The metaphase plates of these cells are indicated

1) Essentials of these accounts were read before the 20th Annual Meeting of the Zoological Society of Japan at Nagoya, October, 1949, and the 22nd Annual Meeting of the Genetic Society of Japan at Tokyo, October, 1950.

in Figures 61 to 63. In Figurea 57 to 59, the successive stages of anaphase showing the course of the separation of chromosomes are illustrated: there occur neither lagging of chromosomes nor chromosome-bridges, nor any other abnormalities such as deficient spindle-formation or aberrant orientation of chromosomes. Figures 23 to 24 represent the anaphasic sister complexes of chromosomes respectively, each of which includes well corresponding sister chromosomes. Thus, there is nothing unusual in separation of the chromosomes during anaphase. Figure 60 illustrates the telophase configuration of tumor cells; no aberrant feature is visible in the course of the formation of daughter nuclei.

Finally, it can be said that the mitosis of tumor cells with ± 40 chromosomes possessing a specific karyotype usually proceeds in almost ordinary manner, no aberrant behavior of chromosomes taking place.

3. Comparison of the chromosomes of ordinary somatic cells and tumor cells

In the next step, the comparison between the chromosomes of somatic cell of the host (*Rattus norvegicus*) and tumor cells is of especial interest, in connection with the question how morphological difference or similarity may occur in the chromosomes. The investigation along this line has been extensive. It should be mentioned that tumor cells of the Yoshida sarcoma show a remarkable host-specificity to white rats in their malignant growth.

On account of the favorable material for cytology a considerable amount of work has been done on the chromosomes of white rats (cf. an atlas of chromosome numbers, by Makino 1951). In germ cells of white rats (the Wistar strain), the author has established 42 chromosomes in diploid, all of which were regarded as of simple rod-type from their superficial appearance (Makino 1942, 1943). Some larger ones, however, are characterized by a globular part forming their inner terminals; the globular part usually shows a slight bending. The other elements appear as straight or sometimes slightly curved rods, tapering towards their inner ends. There is not the slightest evidence for the existence of any V-shaped chromosomes. There is only a slight variation in length, forming a closely graded series and no outstanding element is present (cf. Figs. 11–20 in Makino 1942, and Figs. 19–28 in the 1943 paper).

By the application of a new squash technique, Tanaka (1951) recently investigated the chromosomes of white rats from the Wistar strain in somatic cells from various organs. He established, in the majority of cases, 42 diploid chromosomes in various kinds of somatic cells as shown in Figures 25 to 28. According to him, about 80 percent of the observed cases (410 cells out of a total of 533 cells examined) showed the basic number of 42. The remaining 123 cells showed a varia-

ation of the chromosome number ranging from 36 to 84 (cf. Table 1 in the paper of Tanaka 1951). All of the chromosomes both in regular cells and in cells with aberrant chromosome number, were found all to be of simple rod-type, tapering towards their inner extremities; there was no evidence of the presence of a V- or J-shaped one. So far as the general morphological features of chromosomes are concerned, the chromosomes of somatic cells of the white rat exhibit a striking similarity to those germ cells of the same, and there is no visible difference between them, except the numerical variation occurring in some somatic cells. Metaphase examples are shown in Figures 25 to 28. Figures 29 to 31 are the serial alignments of the paired chromosomes in which no clear distinction of the element into any groups can be made.

The comparison of the chromosomes of either germ or somatic cells with those of tumor cells discloses a marked differentiation between the former and the latter, especially in respect of the V-shaped elements occurring in tumor cells. If now we compare Figures 11 to 22 from tumor cells with Figures 29 to 31 from normal cells, for instance, the morphological difference of chromosomes existing between them is at once very apparent.

As already noted in the foregoing section, the chromosomes of well-balanced subdiploid tumor cells can be divided into two distinct sets, namely the set of rod-shaped chromosomes and that of V-shaped ones. In comparison of the chromosomes of tumor cells with those of normal diploid cells, it is evident that the rod-shaped chromosomes, about 12 in pair, occurring in tumor cells seem to correspond to certain 12 pairs out of the 21 homologous pairs of chromosomes found in ordinary somatic cells of hosts, because of their apparent likeness in morphological detail. In other words, the chromosome set comprising rod-shaped elements in tumor cells may in all probability originate from the white rat host on account of their morphological similarity. However, one fails to find at all any V-shaped elements, in somatic cells of white rats. Obviously, the cells of white rats possess no elements corresponding to the J- or V-chromosomes found in tumor cells. Therefore, it follows, the set of V-elements can be regarded as of the tumor cell proper. At present, the explanation remains difficult regarding the origin and nature of these V- or J-elements found in tumor cells.

From the above observations, it is apparent that the chromosomes of tumor cells are provided with two distinct sets, probably dissimilar in nature; one set consists of rod-shaped chromosomes which seem to originate from the host cell, and the other set is characterized by the J- and V-shaped chromosomes which are specific to tumor cells being unknown as to their origin. On account of these characteristics, the chromosomes of tumor cells show a marked differentiation from those of the host, white rats. So far as the observations go, it is especially

notable that, there has been demonstrated no transitional type of chromosomes between ordinary somatic cells and tumor cells.

4. Observations of the chromosomes of tumor cells through the heteroplastic transplantation

From the above observations it is evident that the tumor cells are chromosomally specialized, because the chromosomes are markedly differentiated from those of the host. In order to understand the individuality or constancy of the chromosomes in these tumor cells, it is important to see whether the chromosomes undergo shifting or whether they remain unaltered in tumor cells due to the heteroplastic transplantation. With this point in mind the following observations carried out in tumor cells transplanted in some heterogeneous animals.

It has been well shown that the Yoshida sarcoma is characterized by tumor cells which display a remarkable host-specificity to white rats in their malignant development (Yoshida 1949). Recently, T. H. Yosida (1952) has shown that tumor cells of white rats transplanted into some heterogeneous animals related to white rats, such as black rats (*Rattus rattus*), white mice (*Mus musculus*), field mice (*Apodemus geisha*), voles (*Clethrionomys bedfordiae*) and guinea pigs (*Cavia cobaya*), could continue to live for a certain period in the peritoneal cavity of these heterogeneous hosts, and showed mitotic division in more or less degree. But, they all disappeared later and the hosts remained alive. The tumor ascites was taken out of these heterogeneous animals a few days after transplantation and smeared with acetocarmine for observation. The tumor cells injected into the peritoneal cavity of white mice underwent a considerable proliferation with a plenty of dividing figures favourable for study of chromosomes, but the transplantation made in other animals resulted in showing only a few dividing figures of tumor cells. For this reason, some extensive studies have been possible in the material derived from white mice.

Figures 32 to 34 illustrate the metahpase chromosomes in tumor cells of the Yoshida sarcoma developed in the peritoneal cavity of white mice. They show the chromosome numbers of 39, 40 and 41, respectively. Nakahara (1952) made some observations on the chromosome numbers in tumor cells transplanted in the mice and reached a just comparable result to the case of the homoplastic transplantation as follows:

Chromosome. Noumbers.	35	36	37	38	39	40	41	42
No. of cells observed	1	3	4	2	5	5	16	1

Viewed morphologically, the chromosomes disclose also a striking similarity to those found in tumor cells from homoplastic transplantation in white rats. The

evidence will be understood more clearly than any verbal descriptions by referring to the chromosomes in serial alignment shown in Figures 35 to 40; the comparison of the latter figures with Figures 11 to 22 from the homoplastic transplantation reveals no significant difference among them. Furthermore, there is again a close similarity of chromosomes between any two chromosome sets within the mouse-transplantation. The similar feature was also met with in other heteroplastic transplantations as described below.

In Figures 41 to 46 are shown the metaphase chromosomes observed in tumor cells which were heteroplastically transplanted into black rats (*Rattus rattus*, Fig. 41), field mice (*Apodemus geisha*, Figs. 42–43), voles (*Clethrionomys bedfordiae*, Figs. 44–45) and guinea pigs (*Cavia cobaya*, Fig. 46), with the chromosome numbers ranging from 38 to 42. The morphological analysis of the chromosomes in these cells was made by means of the serial alignment of supposed mates as seen in Figures 47 to 55. Referring to these figures, the uniformity of chromosomes among the cells from differnt hosts is very striking. It is noticeable that in every case the chromosomes are largely differentiated from those of hosts[1] both in constitution and in number, but are remarkable for comprising specific constitutents characteristic of tumor cells. This fact is at once evident by the comparison of these figures (Figs. 47–55) with Figures 11 to 22 (from homoplastic transplantation); there is again a remarkable morphological similarity of chromosomes between tumor cells derived from homogeneous hosts and those from heterogeneous hosts, not only in their numerical relation but also in morphological respects.

The series of above facts indicates that there are tumor cells in the Yoshida sarcoma which show no significant change of chromosomes through transplantation either into homogeneous animals or into heterogeneous animals; these tumor cells show in both cases a uniform chromosome constitution characteristic of the Yoshida sarcoma, but largely differentiated from that of the host. In other words, the chromosomes observed in certain tumor cells which were introduced into some heterogeneous animals, are essentially similar to those in certain tumor cells from the specific host, white rats. Obviously, this implies that the tumor cells which were introduced into some heterogeneous animals and divided there for some times, are those which come from white rats. Thus, there is no doubt that certain tumor cells of white rats have remained without changing their chromosomal individuality through succeeding transplant generations, and even

1) Concerning the chromosome constitution of the animals described here, refer to Oguma (1935) and Makino (1941, 1943) for mice and black rats, Makino (1951) for field mice, Oguma (1935) for voles, and Makino (1947) for guinea pigs, respectively. The chromosomes of these animals are largely different from those of the tumor cell, not only in number but also in morphological details.

also in cases of the heteroplastic transplantation.

Finally, it can be emphasized in the light of the above findings that there is present in the Yoshido sarcoma an established strain of tumor cells having their own characteristic chromosome complex and maintaining malignancy as well as inherited capacity for autonomous growth; their chromosome individuality has remained unchanged from their original ancestor through transplant generations from host to host. It is these tumor cells that primarily contribute to the growth of the tumor. The cells showing various mitotic abnormalities of common occurrence are evidently derivatives of these strain cells; obviously they are produced due to abnormal mitosis through the alteration of normal spindle mechanism, the structural change of chromosomes and other unknown causes, as mentioned in the former paper (Makino and Yosida 1951; Makino and Kanô 1951). Certainly, these abnormal cells cannot continue division much longer and may degenerate in near future.

Concluding Remarks and Critique

1. Behavior of tumor cells in a transplant generation

In the former studies, morphological observations and the statistical survey of the mitotic abnormalities, the mitotic rate and the variation of the chromosome number have been made in tumor cells of the Yoshida sarcoma through a transplant generation. The conclusion reached was that there are present certain tumor cells with well-balanced subdiploid chromosomes, 40 or thereabouts in number, and ordinary in metaphase configuration, and further that, on account of their definite mitotic rate along with their remarkably high frequency, they participate importantly in the growth of the tumor (Makino and Yosida 1951; Makino and Kanô 1951). The present investigation has revealed that those tumor cells which contribute to the growth of the tumor possess a characteristic chromosome constitution; they are very remarkable and highly differentiated from that of the host, by comprising 22–24 elements of rod-shape and 16–18 elements assuming J- or V-shape of various sizes. Furthermore, the chromosome individuality of these cells has remained unaltered through successive transplant generations from host to host. Obviously, the above findings are a strong indication that there is present a strain of tumor cells with a definite chromosome constitution characteristic of this tumor, and that these tumor cells primarily play a significant role in the growth of the tumor through their regular mitotic multiplication.

As formerly noted, by intraperitoneal introduction the Yoshida sarcoma grows in the new host and leads the diseased animal to death in 12 days on the average. Based mainly on the data concerning the mitotic rate (cf. Table 1 and 2, Charts

1 and 2 in the paper of Makino and Kanò 1951), the behaviour of tumor cells in a transplant generation cycle is described as follows:

Following the transplantation of the tumor, many of tumor cells newly introduced into the new host seem to undergo degeneration, because the cells in process of disintegration and manifesting various abnormalities appear at a high rate. Dividing cells are very few. The majority of the degenerating cells are those of large size; they are characterized by a considerable amount of cytoplasm with a large bilobed or kidney-shaped, or sometimes many-lobulated nucleus (cf. Fig. 24 in Makino and Kanò 1951). The giant cells containing huge or many nuclei are also not uncommon. Obviously these cells are to be considered derivatives from the strain cells, which have received an aberrant chromosome constitution through abnormal mitoses.

The mitotic figures of tumor cells begin to appear at about 24 hours after transplantation (Figs. 64–65). At the same time the number of small cells shows a gradual decrease. It is highly apparent, therefore, that the small cells undergo mitotic division. They show in mitosis a well-balanced subdiploid complex of particular chromosomes characteristic of tumor cells, as seen in Figures 61 to 65. So, it is beyond question that they are the strain cells. The number of mitotic figures of strain cells progressively increases with the passage of time. On the 3rd or 4th day after transplantation, the strain cells show a very active multiplication. Probably the peritoneal fluid of the host at this stage serves as a favourable culture medium for multiplication of tumor cells. Towards the middle part of the life span of the tumor animal, the cells undergoing mitosis attain the highest frequency throughout the transplant generation. Then, they gradually decrease towards the latter part of the life span. In response to the decrease of the mitotic cells, the frequency of cells displaying mitotic abnormalities as well as those in the course of disintegration increase. Nearing the last day of the tumor animal's life, mitotic figures of the strain cells show a sudden decrease in occurrence, while the cells showing abnormalities as well as those in course of disintegration appear at a strikingly high rate. Intermingled with these abnormal cells, there occur in the tumor ascites a number of resting cells of small size, which are characterized by their small amount of cytoplasm and by well-defined compact nuclei (Figs. 66–68). These resting cells correspond in all probability to the strain cells, which fact has been proved by some experiments carried out by Makino and Tanaka (unpublished). By the end of the life span, the accumulation of the tumor ascites reaches an enormous amount resulting in a remarkable expansion of the abdomen of the host. The malignant growth of the tumor having reached this stage leads to the death of the host.

As shown in the above descriptions, the strain cells actively multiply under-

going a regular mitosis during the period from the early to the middle part of the life span in a transplant generation. During the course of the multiplication, a number of the derivatives of these strain cells became abnormal through aberrant mitosis, due to the change of normal spindle mechanism, the structual alteration of chromosomes and some other unknown causes. The cells showing mitotic abnormalities and those in the process of disintegration increase in number with a proportional relation to the accumulation of the tumor ascites which takes place during the latter part of the life of the tumor animal. It is most probable that the accumulation of the tumor ascites, which contains in the greater part degenerative products of tumor cells, brings about the change in viscosity of ascites; this change may result in the disturbance of the water relation within tumor cells. This may be followed by the change of normal spindle mechanism as well as structural alteration of chromosomes due to hydration or dehydration processes, leading to various mitotic abnormalitites.

On the other hand, there are a part of the strain cells which remain without changing their specific chromosome individuality, persisting not only in the characteristic number of chromosomes but also in their particular morphological constitution. In the latter part of the life span of the tumor animal when the ascites probably provides unfavourable condition for cells to continue division, these strain cells remain inactive and persist in the resting stage (cf. Table 1–2, Makino and Kanô 1951); they are characterized by a small amount of cytoplasm and by compact basophilic nuclei (Figs. 66–68). Some experimental examinations[1] relating to permeability of the cell have shown that these small resting cells have acquired a new property in their surface to protect themselves from the unfavourable surrounding medium. When these cells are inoculated into the peritoneal cavity of the new host, they begin multiplication under a new and favourable culture condition, and start another cycle. Thus, it follows that the successive transmission ofthetumor is performed by the strain cells from host to host.

2. Theories concerning the origin of cancer

The present investigation has made it clear that there is present a strain of cells which primarily participate in the growth of the tumor and contain a peculiar chromosome constitution characteristic of the present tumor. That is, these tumor cells contain well-balanced subdiploid chromosomes, 40 or thereabouts in number; the chromosome complex of these cells consists of two remarkable sets, probably

1) For example, with the treatment of 0.25 M $CaCl_2$ solution most of the large tumor cells were destroyed as a result of dehydration, while the small compact cells remained unaffected. This seems to indicate a difference in permeability of the cellular surface between the large- and small-sized cells.

dissimilar in both nature and structure. One set comprises rod-shaped chromosomes probably originating from the host, while the other set comprises J- and V-shaped elements of various sizes, unknown in their origin. On account of this characteristic peculiarity, the chromosome complex of these tumor cells is markedly differentiated from that of the host cell. Previously, there has been no publication on a corresponding finding in the field of cancer research. Winge (1930) described that the cells with the chromosome numbers ranging from 36 to 40 were most frequent during the pre-cancer stage of the tar-induced mouse carcinoma. But, he paid no attention to the constitution of the chromosomes.

For explanation of the peculiar chromosome complex of tumor cells which shows a remarkable differentiation from that of the tissue cells of the host, the occurrence of chromosomal mutation is most probable. It has frequently been said that the origin of cancer must be essentially mutational in nature or in experiment. At some stage in the life history of an individual or in the course of experiment with carcinogens, a cell or a group of cells in the tissue of the normal individual undergoes a mutational change to acquire a capacity for autonomous growth. As the cells multiply, there results a strain of cells with an inherited capacity for autonomous growth. Various hypotheses concerning the origin of cancer point to a somatic mutation in a broad sense. The mutation is; **i**) genic, connected with a gene mutation; **ii**) chromosomal, being a result of a change in the number or structure of chromosomes; **iii**) heterochromatic, depending on a change in the amount of heterochromation, and **iv**) plasmatic, involving the idea that a change has taken place in the so-called plasmagenes. The former three hypotheses are concerned with the change which leads to the development of cancer as having taken place in the chromosomes, while the fourth postulates that the change occurs in the cytoplasm. Since it has early been proposed by Boveri (1914) and some earlier worker (cf. Politzer 1935), the varying chromosome numbers observed in cancer cells have long been assumed to be the cause of their malignancy. In fact, cancer cells display a striking variation of chromosome number; for instance the chromosomes of the Yoshida sarcoma vary in number ranging from about 20 to over 80, or sometimes to high polyploids. But, on the other hand, the chromosome numbers likewise vary greatly in somatic cells of normal tissues; Tanaka (1951) demonstrated that in various tissue cells of young albino rats the chromosomes show a numerical variation which ranges from 36 to 84. On this basis, therefore, the variation of the chromosome number alone can hardly be regarded as the ultimate cause of the malignancy of cancer. The weak point of this hypothesis is that the previous authors have been attracted merely to the numerical change of chromosomes. Turning to the present author's case, however, the situation is highly different; the chromosome complex of the Yoshida

sarcoma cells is remarkable in showing a prominent differentiation from that of the host. That is, roughly half of the members forming the complex are of the host origin, while the other half are of those newly transformed. Moreover, the individuality of chromosomes remains unaltered in these tumor cells through successive transplant generations. Obviously, this event is not a mere numerical change of chromosomes, but a clear-cut evidence illustrating that the cells of this tumor have arisen in connection with mutational change of chromosomes in the ordinary tissue cells. Probably, at some stage in the course of experiment with carcinogenic agents, a cell or a group of cells in the tissue of the normal individual undergoes a mutational change in chromosomes so as to acquire a capacity for autonomous growth. Thus, tumor cells which possess a characteristic complex of chromosomes along with a heritable autonomous capacity and are capable of successive transmission, closely simulate a parasitic organism in behaviour. It is not out of place to draw attention to the experiment on a single cell transplantation made in this sarcoma. Ishibashi (1950) and Hosokawa (1950) reported that the transmission of the Yoshida sarcoma has been successful even with a single tumor cell to some extent (about 60 percent), but without cell the transplantation was impossible.

Recently, Caspersson and Santesson (1942) observed that the nucleic acid metabolism is governed by the heterochromatic regions of the chromosomes, and that malignant cells contain more nucleic acid than normal cells. They postulated that the origin of cancer is to be sought in a change of the heterochromatic portions of the chromosomes. In view of the assumption that the observed morphological change in chromosomes may involve some intrachromosomal alteration, the argument of Caspersson and Santesson (1942) is not to be easily disregarded.

Recent communications published by Koller (1947), Darlington (1948), Timonen and Therman (1950) and some others emphasized that the permanent change which renders a cell malignant takes place in the cytoplasm. There is, however, no direct morphological basis for that assumption, though Koller (1947) argued that, since the cell remains active in spite of its deficient nucleus, mitotic activity is under cytoplasmic and not nuclear control. That this view of Koller (1947) is not applicable for the interpretation of the related phenomenon occurring in the Yoshida sarcoma, was seriously discussed in the former paper (Makino and Kano, 1951). Timonen and Therman (1950) claimed also that the origin of cancer is to be sought in a change occurring in the cytoplasm, because the most constant feature characterising all the cases of cancer is the general acceleration in the division rate which is connected with the acceleration rate of the spindle mechanism. But, by the recent investigation it is strongly indicated that the spindle substance is not cytoplasmic but of the nuclear origin (Wada, 1949, 1950). Obvi-

ously, the latter fact is largely unfavourable for their argument.

In broaer dsense, it seems very probable that the origin of cancer cannot be sought in a single cause, that there are several types of cancer differing in nature and in origin, and that there are several causes in the development of cancer.

Summary

Summing up the results from the former studies (Makino and Yosida 1951, Makino and Kanô, 1951) together with those of the present investigation, it can be concluded that there exists a strain of tumor cells which multiply with a regular mitotic behaviour and participate primarily in the growth of the tumor. These tumor cells possess a peculiar chromosome complex characteristic of this tumor, not only in the number of chromosomes but also in their morphological features. They contain well-balanced subdiploid chromosomes, 40 or thereabouts in number; the chromosome complex is provided with two distinct sets, probably dissimilar in both structure and nature. One of the sets consists of rod-shaped chromosomes ranging from 22 to 24 in number; they seem to originate from the cells of the host on account of their morphological likeness. The other set comprises J- and V- elements of varying sizes, about 16 to 18 in number, which are unknown in their origin because there are no corresponding elements in the host cells. On account of this characteristic peculiarity, the chromosomes of these tumor cells are markedly differentiated from those of the host cells. Furthermore, there has been demon strated no transitional type of chromosomes between normal cells and tumor cells.

The individuality of chromosomes in the strain cells of the tumor remains unchanged through successive transplant generations from host to host. The tumor cells showing mitotic abnormalities of common occurrence are evidently derivatives from these strain cells; they were produced through abnormal mitosis due to the alteration of normal spindle mechanism, the structural change of chromosomes and other unknown causes. On the basis of the above findings, the behavior of tumor cells through a transplant generation have been described in the present paper.

Critique was offered on various hypotheses previously proposed concerning the origin of cancer. The peculiar chromosome complex in tumor cells which shows striking differentiation from the complex in the ordinary tissue cells, together with its heritable capacity for autonomous growth, is well explicable by taking the view that the tumor cell has arisen due to a kind of mutation from the tissue cell. Probably, at some stage in the course of certain experimental treatment, a cell or a group of cells in the tissue of the normal individual undergo a mutational change in chromosomes so as to acquire a capacity for autonomous growth. Thus, the tumor cells, which possess a characteristic complex of chromosomes

along with a heritable autonomous capacity and are capable of successive transmission, closely simulate a parasitic organism in their behavior.

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Explanation of Plate IX

All are camera-lucida drawings. 1–10, 23–25; ca. ×1300.

Figs. 1–6, metaphase plates of tumor cells showing well-balanced subdiploid chromosomes; 38, 40, 40, 41, 42 and 43 chromosomes in each. **Fig. 7,** metaphase plate showing 35 chromosomes. **Fig. 8,** metaphase plate showing 26 chromosome. **Fig. 9,** metaphase plate of a subtriploid cell, showing 64 chromosomes. **Fig. 10,** metaphase plate of a subtetraploid cell, showing 81 Chromosomes. **Fig. 11–22,** serial alignments of supposed homologous pairs of chromosomes in descendant order, showing the composing elements of well-balanced subdiploid tumor cells (strain cells). In each, 22–24 elements are rod-shaped and 16–18 chromosomes are V- or J-shaped. **Fig. 23,** regular behavior of chromosomes at anaphase of a well-balanced subdiploid cell (strain cell). **Fig. 24,** telophase of the same, showing regular separation of chromosomes. **Figs. 25–28,** chromosomes of normal diploid (somatic) cells of white rats (Wistar strain). 25, blood cell. 26, spermatogonial cell. 27, liver cell. 28, brain cell. 42 chromosomes in each.

Explanation of Plate X

All are camera-lucida drawings. 32–34, 41–46, ca. ×1300.

Figs. 29–31, serial alignments of supposed homologous pairs of ordinary somatic chromosomes in descendant order. 29, blood cell. 30, liver cell. 31, amnion cell. **Figs. 32–34,** metaphase plates of tumor cells transplanted in mice (*Mus musculus*); 39, 40 and 41 chromosomes in each. **Figs. 35–40,** serial alignments of supposed homologous pairs of chromosomes in tumor cells transplanted in mice. 22–24 elements are rod shaped and 16–18 V- or J-shaped. **Fig. 41,** metaphase plate of a tumor cell transplanted in black rat (*Rattus rattus*), showing 38 chromosomes. **Fig. 42–43,** metaphase plates of tumor cells transplanted in field mice (*Apodemus geish*), showing 40 and 41 chromosomes, respectively. **Fig. 44–45,** metaphase plates of tumor cells transplanted in voles (*Clethrionomys bedfordiae*), 42 chromosomes in each. **Fig. 46,** metaphase plate of a tumor cell transplanted in guinea pig (*Cavia cobaya*), showing 42 chromosomes. **Fig. 47–55,** serial alignments of supposed homologous pairs of chromosomes in tumor cells, transplanted in various heterogenous animals. 22–24 elements are rod-shaped and 16–18 elements V- and J-shaped. 47–49, from guinea pig-transplantation. 50–52, from vole-transplantation. 53–54, from field mouse-transplantation. 55, from black rat-transplantation.

Explanation of Plate XI

All are photomicrographs of tumor cells. (*S. Makino photo.*)

Figs. 56–60, successive stages of mitotic division of well-balanced subdiploid cells (strain cells), showing regular behavior of chromosomes in the course of separation. (×1200). **56,** late prophase. **57–58,** anaphases. **59,** telophase. **60,** separation of the cell body. **Figs. 61–63,** metaphase plates of well-balanced subdiploid tumor cells forming a strain, from the tumor ascites at about 20 hours after transplantation (×1800). **Figs. 64–65,** a group of tumor cells

showing a dividing cell, from the tumor ascites at about 20 hours after transplantation. (×900).
Fig. 66, tumor cells of varying sizes, showing abnormal large cells together with the strain cells of small size. From the tumor ascites at a few hours before the death of the host. (×400).
Figs. 67–68, tumor cells of varying sizes, including the small strain cells, indicated by arrows. From the tumor ascites at the times of the death of the host. (×600).

Figs. 56–63, 66: acetocarmine preparations.
Figs. 64–65, 67–68: Giemsa preparations.

摘　　要

癌の細胞学的研究，第 III 報　吉田肉腫腫瘍の生長に関與する腫瘍細胞の核学的特性ならびに固有性

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第 I 報及び第 II 報においてなされた腫瘍細胞の移植一世代を通じての分裂ならびに染色体に関する基礎的調査の結果からして，吉田肉腫の移植一世代における腫瘍細胞の増殖には，正規な分裂行動をとり，二倍数に近い染色体数をもった一群の細胞が主体となっていることが明らかになった。それらの細胞はシロネズミの体細胞とは明らかに異る固有の，しかも一定した核型をもっている。即ち，シロネズミの体細胞は 42 個の棒状染色体をもっているが，これらの腫瘍細胞は 40 前後の染色体数をもち，22~24 個の棒状染色体と，16~18 個の J 型ならびに V 型の染色体より成る核型をもっている。このように吉田肉腫には腫瘍固有の染色体型をもった腫瘍細胞の一群の種族があって，それらの細胞の固有性は累代移植を通じて変化することなく保たれ，連綿として細胞から細胞えと傳えられる。この腫瘍細胞の固有性はシロネズミ以外の動物に異種移植した場合にも変化しない。腫瘍の生長に第一義的に主要な役割をなすものは，これらの一群の腫瘍細胞の種族であって，宿主の組織細胞が一時的にそれに與っているものではない。そのことは，累代移植の如何なる時期の腫瘍細胞においても，異種移植の場合においても，また如何なる個体からとった材料においても，腫瘍細胞が常に一定の而も特有の核型をもっていることから，自ら明らかである。吉田肉腫において累代移植の源をなすものは，腫瘍細胞として変化をうけた一群の細胞の種族であって他の何物でもない。これらの細胞は，恐らくこの腫瘍がつくり出される過程において，何等かの原因によりシロネズミの或る組織細胞が変化を起して生じたものであって，病的な自律性を獲得し，一群の腫瘍細胞の種族として発達したものであろう。これらの細胞種族は種族保存の能力を有し，累代移植においてその固有性を保持するばかりでなく，各種の化学薬品とか X 線などに対しても強い抵抗性を有し，たとえそれらによって処理をうけても，少数の種族細胞は破壊を免かれて生存し，再び腫瘍を再発する。吉田肉腫にも他の腫瘍と同じく，腫瘍細胞にはいろいろな種類の分裂の異常が発見される。これらの異常分裂は種族細胞の或るものが変性して生じた結果である。異常分裂をして核内容の異常となった細胞は早晩死滅の運命にあるもので，永く分裂を継続する可能性はないから，癌のように急激に生長するものにはそれに與って重要な役割を演ずるものとは考えられない。　(文部省科学研究費補助)


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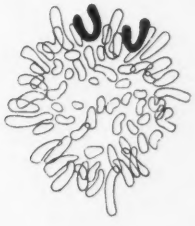
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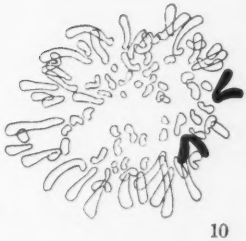
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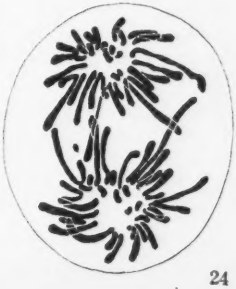
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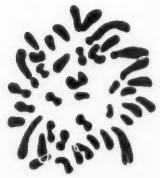

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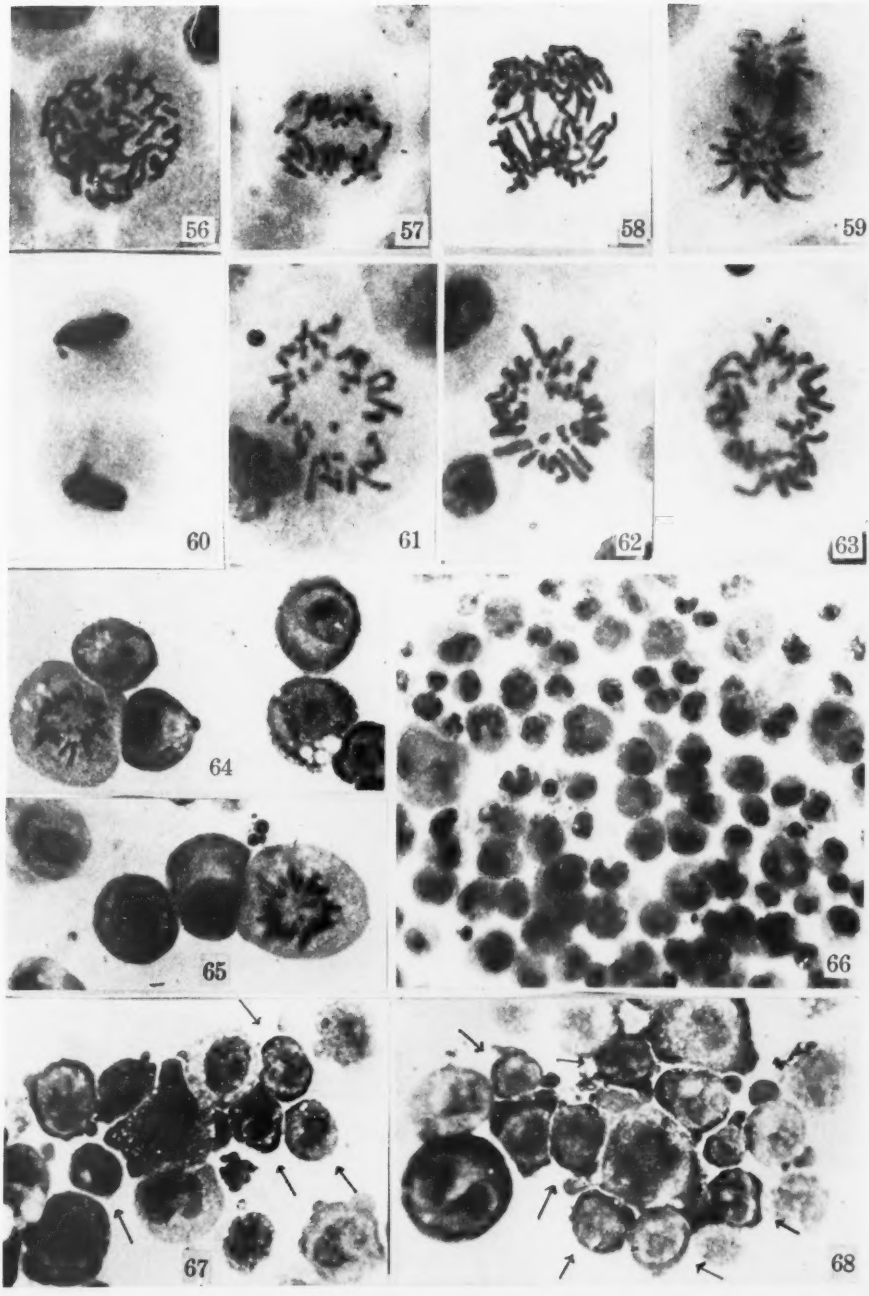
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[GANN, Vol. 43, April, 1952]

CYTOLOGICAL STUDIES ON CANCER. V. HETEROPLASTIC TRANSPLANTATIONS OF THE YOSHIDA SARCOMA, WITH SPECIAL REGARD TO THE BEHAVIOUR OF TUMOR CELLS[1]

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Experiments of heteroplastic transplantations in tumors have long attracted attention in the field of cancer pathology and a considerable number of reports have been produced along this line in both mammals and birds (Seiffert, 1925, Woglom, 1929, Ligneris, 1932, Andrewes, 1932, Oshima, 1938, Okamura & Namiki, 1938, Nagayo, 1941, Yoshida, Wakahara & Osada, 1948, Shimanouchi & Suwa, 1948, Makino, 1949 and Tatsumi, Nagatomo & Shibano, 1949). It has been shown that the Yoshida sarcoma here concerned is an ascites tumor specific to white rats (*Rattus norvegicus*), characterized by tumor cells which show a remarkable host-specificity in their malignant development. Studies of the heteroplastic transplantations of the Yoshida sarcoma have been undertaken by Yoshida et al. (1948), and Shimanouchi & Suwa (1948) into mice, Makino, Y. (1949) into guinea-pigs, Tatsumi et al. (1949) into rabbits and mice. These studies have been worked out with animals taxonomically related to white rats, mainly with the purpose to ascertain heterologous transplantability of tumors. In the present study, the author aimed to observe the behavior of tumor cells of the Yoshida sarcoma in heteroplastic transplantations concerning the following animals; *Rattus rattus, Mus Musculus, Apodemus geisha, Clethrionomys bedfordiae, Cavia cabaya, Eutamias asiaticus lineatus, Lupus cuniculus domesticus, Felis domestica* and *Gallus gallus domesticus*.

It is the author's pleasant duty to express his gratitude to Professor Sajiro Makino, under whose suggestion and guidance the work has been carried out, for his keen interest and for his improvement of this manuscript.

MATERIAL AND METHODS

Animals used as heterologous hosts in the present experiment are the following

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This article constitutes one of the serial studies having been done by S. Makino and his co-workers (S. M.).

nine forms which cover 7 species of Rodentia, 1 species of Carnivora and 1 species of birds.

Rodentia	Muridae	*Rattus rattus* (2)*, *Mus musculus* (4), *Apodemus geisha* (1), *Clethorionomys bedfordiae* (1)
	Cavidae	*Cavia cabaya* (3)
	Sciuridae	*Eutamias asiaticus lineatus* (2)
	Lepolidae	*Lupus cuniculus domesticus* (2)
Carnivora	Felidae	*Felis domestica* (1)
Galli	Phasianidae	*Gallus gallus domesticus* (1)

The transplantation of the tumor was performed with the aid of the glass pipette usually used for the purpose of successive transmissions of this sarcoma. Microscopical observations of tumor cells were made with the smear preparations according to Giemsa's technique. Details of the method of transplantation and of the microscopical technique are described in the former paper (Makino & Yosida, 1951). Daily observations were continuously made beginning on the next day after transplantation of the tumor.

Result of Observations

1. Behavior of tumor cells

A). Homoplastic transplantation (a control experiment)

The tumor cells transplanted homoplastically in a white rat (*Rattus norvegicus*) showed the typical behavior as the Yoshida sarcoma, in accordance with the results presented by Makino and Kanò (1950). The rat which received the tumor died on the nineth day after transplantation.

B). Heteroplastic transplantations

a). *Rattus rattus:* In one of the individuals studied here, the tumor cells after inoculation showed mitotic proliferation during a period of four days, while in the other individual the proliferation of tumor cells continued during the first two days after transplantation. Afterward the number of tumor cells suddenly decreased in every case. Then the tumor cells completely disappeared from sight in the ascites of these animals.

b). *Mus musculus:* Four specimens were used in transplantation. Two of them underwent the transplantation of the tumor directly from white rats. In both individuals the tumor cells showed considerable proliferation for a period of six days after transplantation. The dividing cells exhibited rather regular mitotic behavior. In the other two mice which received the tumor by transmission from

*Numerals in parenthesis indicate the number of individuals employed in experiment.

the former two mice, the behaviour of tumor cells was somewhat different from that observed in the former two cases. In these specimens the proliferation of tumor cells took place for four and five days, respectively, and the majority of dividing tumor cells furnished various types of abnormalities, the healthy mitotic cells being very few.

The results from the above experiments regarding the mouse-transplantation suggest that the tumor cells, in the case of heteroplastic repeated transplantation, seem to behave more vigorously in cass of direct transmission than in the indirect transmission.

c). *Apodemus geisha:* The tumor cells continued to divide in the ascites of this animal for a period of four days after transplantation. Then the proliferation of cells stopped, and the disintergration of cells followed.

d). *Clethrionomys bedfordiae:* Multiplication of tumor cells took place for four days in this animals. But on the fifth day the ascites showed no tumor cells.

e). *Cavia cabaya:* Of three specimens coming under study, two individuals showed the tumor cells which continued to proliferate in the ascites for three days after transplantation. Then they began to degenerate. In the remaining one individual, the tumor cells inoculated had no mitotic activity.

f). *Eutamias asiaticus lineatus:* Some tumor cells together with a few dividing cells were met with in the peritoneal cavities of two specimens studied for two and three days after transplantation, respectively. The cells were observed in stages of degeneration.

g). *Lupus cuniculus domesticus:* Two individuals here under observation were nearly similar in respect to the behavior of tumor cells. A few such cells were observed undergoing degeneration in the ascites, without including any mitotic cells.

h). *Felis domestica:* In the present case, observations of the ascites in the usual way failed to demonstate tumor cells at all. But by centrifuging the ascites a few tumor cells were detected; they were all in stages of pronounced degeneration.

i). *Gallus gallus domesticus:* The demonstration of tumor cells was completely impossible in this case, because of the difficulty in obtaining the ascites owing to its extremely small amount.

Remarks: Based on the above data obtained in the present experiment, it can be said that in the cases of heteroplastic transplantation, tumor cells show different affinity to the different hosts according to their taxonomical relationship. Generally speaking, in the animals systematically close to the white rat (*Rattus norvegicus*), tumor cells transmitted therein continue to live and proliferate in good condition for at least several days in the peritoneal cavities of heterologous hosts. The evidence has been clearly furnished in the heteroplastic transplantations concerning *Rattus rattus*, *Mus musculus*, *Apodemus geisha*, and *Clethrionomys*

bedfordiae. But, the case of the guinea-pig transplantation forms an exception; while the guinea-pig is systematically rather far from the white rat, the tumor cells transmitted therein proliferate to a considerable extent with an active division. On the other hand the tumor cells transmitted in the squirrel (*Eutamias asiaticus lineatus*) show no active mitotic multiplication, though the squirrel is rather nearer to the rat than is the guinea-pig. Similar evidence was met with in the case of the rabbit-transplantation; the tumor cells inoculated in the rabbit's body undergo degeneration without showing mitotic division. In the perioneal cavities of the cat and also of the fowl the tumor cells seem to be unable to continue living. Thus, in every case studied here the tumor cells inoculated into the heteroplastic hosts proceeded to final degeneration, without giving rise to a malignant development of the tumor.

Undoubtedly, the viability of tumor cells in the above mentioned cases is to be regarded as largely connected with the physiological condition of the peritoneal cavity of the host. Probably, the physiological kinship of the body must correspond to the taxonomical kinship of the animal.

II. Mitotic rate of tumor cells in heteroplastic transplantations.

The results of the foregoing observations indicate that tumor cells transmitted heteroplastically in the peritoneal cavities of *Rattus rattus*, *Mus musculus*, *Apodemus geisha*, *Clethrionomys bedfordiae* and *Cavia bavaya*, continue to live for several days and divide showing healthy mitotic behavior. In the following the results of observations on mitotic rate of tumor cells in heteroplastic transplantations will be described, by way of comparison with those obtained in the homoplastic transplantation.

The mitotic frequencies of tumor cells in the cases of both heteroplastic and homoplastic transplantations are given in Table 1. By reference to the data presented there, it is evident that total frequencies of mitotic cells are generally higher in the heteroplastic than in the homoplastic transplantation. This result is attributable to the fact that the number of metaphase cells is strikingly larger in the cases of heteroplastic transplantations than in the homoplastic. The cells in both prophase and anaphase stages show no pronounced difference in number between the two kinds of transplantation. Recently Therman and Timonen (1950) who studied cytologically the tumor of genital organs of women reported that the occurrence of metaphasic cells was higher in malignant tumors than in normal tissues. The present author examined the effect of colchicine on the division of tumor cells in the Yoshida sarcoma and reached the conclusion that cells in the metaphase stage showed remarkably high frequency in occurrence (Yosida, 1951). There is no doubt that the cause of high frequency of metaphasic cells is attributable to the inhibition of the spindle formation in the coures of cell division. It

Table 1. Mitotic rates of tumor cells in heteroplastic transplantations.

Stages \ Hosts	*R. n.*	*R. r.*	*M. m.*	*A. g.*	*C. b.*	*C. c.*
Total No. of cells observed	6600	4264	6118	2812	4014	3011
No. of dividing cells	1.07%	2.89	1.85	2.11	2.10	1.62
Prophase	0.21	0.70	0.24	0.39	0.39	0.36
Metaphase	0.60	1.45	1.20	1.41	1.04	1.12
Anaphase	0.25	0.68	0.42	0.31	0.67	0.13

Remarks. *R. n.=Rattus norvegicus, R. r.=Rattus rattus, M. m.=Mus musculus, A. g.=Apodemus geisha, C. b.=Clethrionomys bedfordiae, C. c.=Cavia cabaya.*

Table 2. Total frequency of mitotic abnormalities in tumor cells observed in heterologous hosts.

Cell types \ Hosts	*R. n.*	*R. r.*	*M. m.*	*A. g.*	*C. b.*	*C. c.*
Division type	36.8	18.7	28.6	21.6	32.4	27.0
Aberrant type	26.0	57.3	27.0	25.4	49.6	40.8
Hypo- & hyperploid cells	1.3	5.6	2.4	1.5	1.9	3.3
Multinucleated & multipolar cells.	3.8	4.1	3.2	1.5	2.3	1.8
Irregular distribution of chroms. at anaphase; lagging and non-disjunction	6.8	27.9	10.3	13.4	17.3	21.9
Displacement and abnormal orientation of metaphase chroms.; hollow metaphase	10.7	11.1	6.3	8.7	13.5	8.2
Deformation of chroms.	0.4	3.0	0.4	0.5	0.4	0.2
Slight stickiness of chroms.; chromosome-bridges	3.0	5.6	4.4	10.3	14.2	5.4
Disintegration type	37.2	23.7	44.2	42.7	17.7	32.2
Total No. of cells observ.	236	197	477	194	259	252

Remarks. *R. n.=Rattus norvegicus; R. r.=Rattus rattus; M. m.=Mus musculus; A. g.=Apodemus geisha; C. b.=Clethrionomys bedfordiae; C. c.=Cavia cabaya.*

is likly that high frequency of metaphasic cells in the cases of the heteroplastic transplantations may be explicable on the basis of a similar cause.

III. Frequencies of abnormal nuclear divisions.

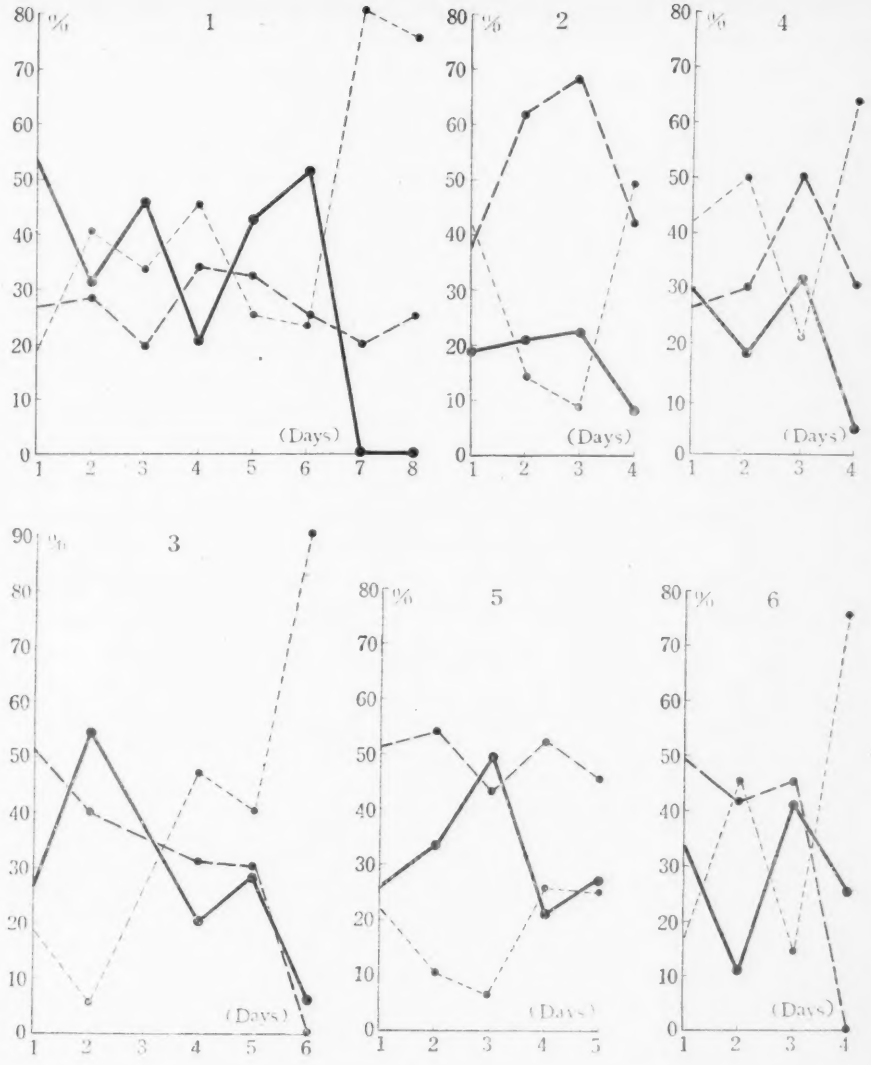
Various mitotic abnormalities observable in the Yoshida sarcoma have been described in the fomer paper, where they were grouped into three types, such as division type, aberrant type and disintegration type (Makino & Yosida, 1951). In this study abnormal mitoses occurring in tumor cells were observed in both heteroplastic and homoplastic transplantations, and the data were arranged in Table 2 according to the different types mentioned above. Generally speaking, the occurrence of division type in tumor cells is higher in the homoplastic transplantation than in the heteroplastic transplantation. On the contrary, the aberrant type showed a lower frequency in the homoplastic transplantation than in the heteroplastic. The disintegration type showed no remarkable difference between the heteroplastic and homoplastic transplantations.

Polyploid cells were frequent in the cases of the heteroplastic transplantations, especially in the cases concerning *Rattus rattus* and *Cavia cabaya*. In the colchicin experiment the author (1951) has found that polyploid and polynucleated cells exclusively increase in appearance within a certain period of recovering from the influence of the drag. The phenomenon may be due to a temporary arrest of the spindle formation. The similar condition seems to occur also in the case of the heteroplastic transplantation. Polypolar divisions and polynucleated cells were infrequent in every transplantation. Stickiness of chromosomes in more or less degree appeared at considerably high frequency especially in the case of *Apodemus* and *Clethrionomys*.

The daily frequencies of mitotic abonormalities in tumor cells showed no remarkable difference between the homoplastic and heteroplastic traesplantations, so far as the present observations are concerned (Figs. 1–6). Generally speaking, the cells of the division type appeared at high frequency during the period from the early part to the middle part of the life span of the tumor rat, decreasing towards the latter part. On the contrary, the cells of the disintegration type were few in number in the early part of the life span. They gradually increased with time, and showed a high frequency in the latter part. The aberrant type, on the other hand, showed some variations with the animals used. The evidence may be understood by reference to Figures 1 to 6.

Summary

The behavior of tumor cells of the Yoshida sarcoma was observed in some heteroplastic transplantations concerning *Rattus rattus, Mus musculus, Apodemus geisha,*



Figs. 1-6. Graphs showing daily frequencies of mitotic abnormalities in tumor cells observed in heterologous animals. Along the abscissa the living duration (days) of tumor cells in heterologous hosts is represented; frequencies of mitotic abnormalities (%) in tumor cells are shown along the ordinate. The solid lines, the broken lines and the dotted lines denote the frequencies of the division types, the aberrant types and the disintegration types, respectively. 1, ***Rattus norvegicus***; 2, ***Rattus rattus***; 3, ***Mus musculus***; 4, ***Apodemus geisha***; 5, ***Clethrionomys bedfordiae***; 6, ***Cavia cabaya***.

Clethrionomys bedfordiae, Cavia Cabaya, Eutamias asiaticus lineatus, Lupus cuniculus domesticus, Felis domestica and *Gallus gallus domesticus.*

The tumor cells could continue to live for a period of several days and divide with healthy mitotic behavior in the peritoneal cavities of the following five forms, *Rattus rattus, Mus musculus, Apodemus geisha, Clethryonomys bedfordiae* and *Cavia cabaya.* Then the tumor cells showed degeneration without progressing to a malignant development of the tumor.

The frequency of occurrence of mitotic cells was higher in the heteroplastic transplantations than in the homoplastic transplantation. The finding is attributed to the fact that the number of metaphasic cells is more frequent in the heteroplastic transplantations than in homoplastic transplantation.

Mitotic abnormalities of tumor cells were observed in the heteroplastic and homoplastic transplantions by way of comparison. The occurrence of the cells of division type was higher in the homoplastic transplantation than in the heteroplastic. The cells of the aberrant type were lower in frequency in the homoplastic than in the heteroplastic transplantations. Those of the disintegration type show no pronounced difference between the two cases.

The daily frequencies of mitotic abnormality compared between the homoplastic and heteroplastic transplantations showed no apparent difference.

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要　　約

癌の細胞学的研究，第V報．吉田肉腫の異種移植，特に腫瘍細胞の行動について

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吉田肉腫を2頭のクマネズミ (*Rattus rattus*)，4頭のハツカネズミ (*Mus musculus*)，1頭のヒメネズミ (*Apodemus geisha*)，1頭のエゾヤチネズミ (*Clethrionomys bedfordiae*)，3頭のテンヂクネズミ (*Cavia cabaya*)，2頭のシマリス (*Eutamias asiaticus lineatus*)，2頭のウサギ (*Lupus cuniculus domesticus*)，1頭のネコ (*Felis domestica*)，1羽のニワトリ (*Gallus gallus domesticus*) の腹腔内に移植して，腫瘍細胞の行動を追求した。

異種の動物は，いかなる種類でも吉田肉腫によって死亡することはない。しかしながら，クマネズミ，ハツカネズミ，ヒメネズミ，エゾヤチネズミ及びテンジクネズミの5種の動物に対しては，ある一定の期間だけ，あたかも同種移植（シロネズミへの移植）の場合の如く，腫瘍細胞は分裂増殖を示した。その他の種類の動物における腫瘍細胞の行動等から，一般的に吉田肉腫細胞の異種動物に対する親和性は，動物の類縁関係と密接な関係のあることがわかった。

腫瘍細胞の分裂増殖をみた5種の動物において，細胞分裂の出現頻度を調べてみた。一般的にいうと，分裂像の出現率は同種移植よりも異種移植の場合の方が高頻度となって表われている。これは異種移植の場合の方が中期核分裂像の出現率が高いためであった。

次に腫瘍胞細の異常性を比較研究した。一般に分裂型細胞の出現率は同種移植の場合の方が高く，これに反して，異常型は異種移植の方が高くなっている。崩壊型は動物の種類によって夫々差異がある。これら異常性の出現率を移植後の経過日数によって調べてみると，その変化の傾向は異種移植及び同種移植の両者の場合において著しい差異はない。即ち，分裂型は初期から中期にかけて高く，後期において著しく低くなっている。これに反して崩壊型は後期に著しく高くなっている。異常型は日によって余り顕著なる上下はない。（文部省科学研究費補助）

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A CASE OF DOUBLE MALIGNANT TUMORS, CARCINOMA OF THE PROSTATE AND THE URINARY BLADDER IN A SINGLE PATIENT

(With Plates XII and XIII)

SHINJI ITO

(From the Department of Urology, Under the Direction of Prof. T. Kusunoki, School of Medicine, Niigata University)

Recently in one patient, 67 year old male, I noticed preoperatively a carcinoma of the prostate and at the same time a small tumor at the dome of the urinary bladder, and the pathological examination of the specimen removed by total cystectomy revealed the adenocarcinoma of the prostate and the transitional cell carcinoma of the urinary bladder as well.

The duplication of primary carcinoma of the prostate and the urinary bladder in the same patient has, as far as I know, not been reported in Japan.

Case Report

K. K., 67 year old male, was sent to the hospital of our department on January 20, 1951, with the complaint of a terminal pain in urination.

His past history revealed that he had gonorrhea at the age of 25, recurring gastric disturbances 25 to 40, and that he had received an operation for urethral calculi at 40.

Since the middle of October 1950, he had been suffering from a terminal pain in urination and when his lower abdomen was pressed a pain extending over the perineum and the glans, was felt, greater in walking. He had never experienced hematuria and the change of the stream. He voided about six times in the daytime and twice in the night time. He had a poor appetite.

The examination revealed a man poorly nourished; 56.5 kg. in weight; temperature 36.6° C; pulse 80, regular but weak in tension; and blood pressure, 114/80. The heart and the lung were normal. The abdomen was soft. Both kidneys could not be felt. The suprapubic area was slightly tender. On rectal examination the right lobe of the prostate was found hard but not so enlarged, which suggested carcinoma. The external genitals were normal. Residual urine was negative.

Cystoscopic examination: The capacity of the bladder was over 300 cc; at the

dome was attached a tumor with a pedicle not so broad and surrounded with areas of edema and slight hyperemia. The excretion of indigocarmin from both urethral orifices was normal.

Laboratory data on admission into the hospital were as follows: Urine—acid, albumin plus, glucose, urobilin and urobilinogen absent, no cast, red and white blood cells plus one. Blood—Hemoglobin 88% (Sahli), red blood cells 3,810,000, white blood cells 6,800, neutrophils 62% with 9% bands, eosinophils 3%, monocytes 1%, lymphocytes 25%. E. S. R. 5, 14 and 90 mm for 1, 2 and 24 hours, respectively. Blood urea nitrogen, 48 mg dl. Wassermann, negative.

An intravenous urogram showed the normal upper urinary tract. The cystogram showed no distinct filling defect. The urethrogram as well showed no remarkable change except the slightly elongated posterior urethra.

The case was clinically diagnosed as the tumor of the urinary bladder and that of the prostate.

After admission into the hospital, his general condition of health was bad and required frequent transfusions of blood.

An operation was performed by Prof. Kusunoki on January 29. On operation, the right half of the prostate was found very hard, not so enlarged, adherent to the surrounding tissues and suggestive of carcinoma. As for the bladder, the wall at the roof where a tumor had been cystoscopically observed was rather thickened. Thus total cystectomy was decided. Both ureters were normal and regional lymphnodes were not swollen. The ureters was anastomosed to the sigma, the right by the method of Kerr-Colby and the left by that of Coffey I.

On macroscopic observation of the specimen removed, there was a tumor of the size of a small finger tip, rising with a pedicle not so broad at the dome of the bladder (Fig. 1). The wall at that part was a little hard but there was scarcely any infiltration at the surroundings. The left lobe of the prostate held several stones of the size of a pin head. The right lobe at the posterior wall near the apex was very hard.

The histological observation, by Prof. Akazaki, Department of Pathology, School of Medicine, Niigata University, was as follows: 1) Urinary bladder: The portion, where tumor had been seen macroscopically was without normal epitheliums, showed ulceration, and was occupied with solid carcinomatous tissue which infiltrated considerably into the depth (Fig. 2). In the cell-nests of carcinoma the arrangement of the cells was quite disordered. Nuclei were generally vesicular and large containing not a little chromatin. Karyomitosis was not marked (Fig. 3). Carcinoma cells were seen partly to have infiltrated into the lymphatic vessels. There were seen in the depth adjacent to the carcinoma the infiltration of round cells mainly composed of lymphocytes and the dilation of blood vessels.

The further microscopic observation of sections showing the parts of the bladder epithelium other than those of the tumor revealed that the bladder epithelium was nearly normal in some part, while in the other the cells were transformed into the type of basal cells extending considerably into the depth; or it sustained formations of Brunn's cell-nests and cysts and vacuolization which are considered to be the feature of the transition into the carcinoma (Figs. 4, 5 and 6). and that it was partly eroded. 2) Prostate: The greater part of the right lobe underwent carcinomatous change. The carcinoma cells were identified as the cuboidal epithelium, forming a small adenoidal structure. Protoplasm was clear. Nuclei were small as those of lymphocytes and rich in chromation. There was little karyomitosis (Fig. 7). Carcinoma cells, close to the back of the prostate, showed the appearance of scirrhus, where several glands with small lumina infiltrated, in a group, into the interstitium. Near the surrounding fatty tissue they were beginning to intrude into the perineurial lymphatic space. Such tissue of carcinoma permeated itself actively into normal acini of the right lobe (Fig. 8). In the anterior of the left lobe, many of the acini had become papillary, and their epitheliums were many-layered and partly formed solid cellnests, which were not yet proliferous to the surroundings and therefore considered to be not a cancer, but a metaplasia. Besides, in the left lobe, there were glands which were dilated and contained desquamated epitheliums and the concrete, but no carcinomatous change was observed. The pathological diagnosis of transitional cell carcinoma of the bladder and adenocarcinoma of the prostate was confirmed.

In spite of the operation performed most adequately the patient was little improved in his appetite and grew weak by degrees. Ascarides often came out of his mouth and anus. Despite of all our efforts for the improvement of his general condition he died on March 29. On autopsy many gray white metastatic tumors on the spine, sternum and ribs were found.

Summary

A case of carcinoma of the urinary bladder accompanied with carcinoma of the of the prostate has been reported here for the first time in Japan.

Note: I have to acknowledge my deep gratitude to Professor T. Kusunoki and Professor K. Akazaki for continuous and cordial instruction in the preparation of this paper.

要　旨

前立腺癌に膀胱癌を併発せる一重複悪性腫瘍例

伊　藤　泰　二
（新潟大学医学部泌尿器科）

患者は 67 才男子。終末時排尿痛，陰茎，会陰部等に枚散する下腹部痛を主訴として來院。血尿，尿線異常等はこれを認めなかった。直腸診で前立腺右葉は硬靱に触れ，しかも余り増大しておらず，癌腫を疑わしめ，更に膀胱鏡的に膀胱頂部にも腫瘍を認めた。膀胱全剔除術により膀胱，前立腺，精嚢を一塊として剔出し両側尿管をS状腸に吻合した。剔除標本を病理学的に檢査して見た所，前立腺右葉の硬靱な部分は明瞭な腺癌の像を示し，膀胱頂部の小指頭大の腫瘍は單純癌（移行細胞癌）であった。更に標本を廣範囲に亘り檢索した結果，後者では前癌性変化に陥った粘膜上皮群からの移行像を証明し，前立腺癌とは明かに別個に無関係に發生していることを確め得た。本症例の如き前立腺癌に膀胱癌の併發例は本邦においては未だその報告をみていない。

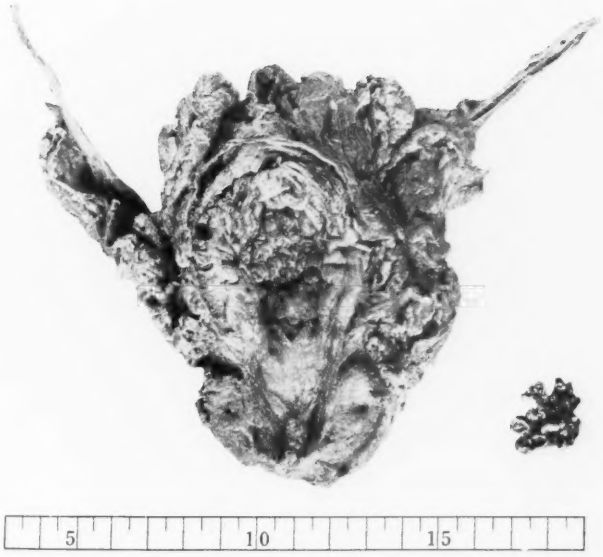

Fig. 1. Gross specimen split open showing a tumor of the dome, and calculi plucked off from the left of the prostate.

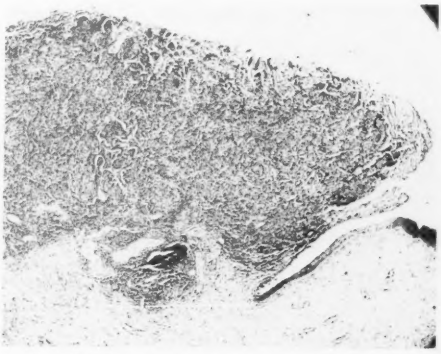

Fig. 2. Showing the solid tissue of transitional cell carcinoma of the bladder (low power)

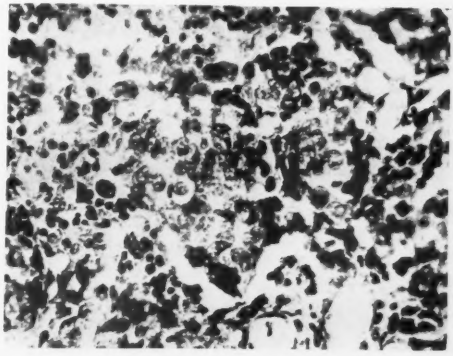

Fig. 3. Showing transitional cell carcinoma of the bladder (high power).

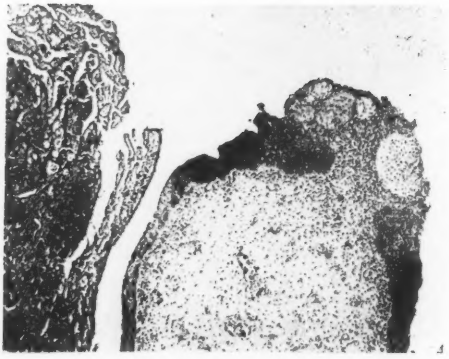

Fig. 4. Showing Bruun's cell-nests near the tumor. On the left, tumor tissue is seen (low power).

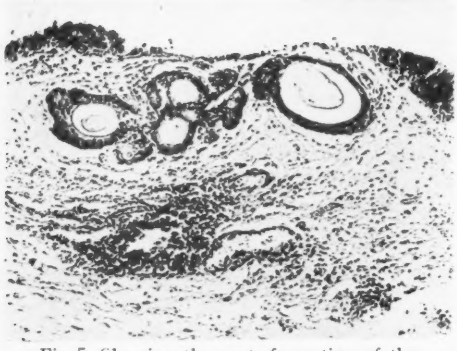

Fig. 5. Showing the cysts-formation of the macroscopically normal epithelium of the bladder (low power).

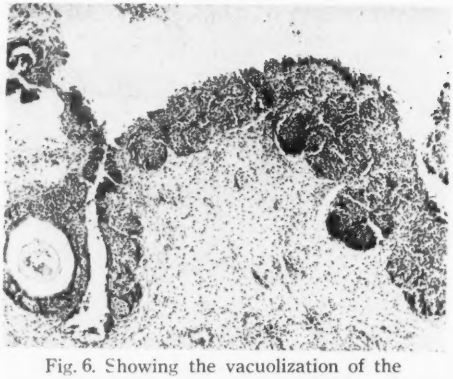

Fig. 6. Showing the vacuolization of the macroscopically normale epithelium of the bladder (low power).

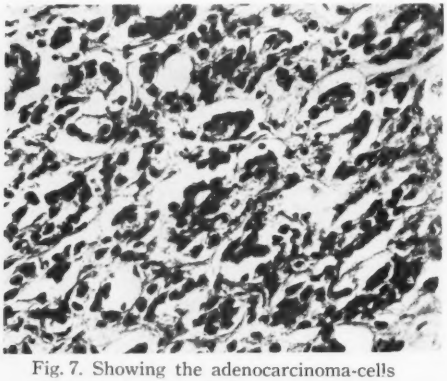

Fig. 7. Showing the adenocarcinoma-cells of the prostate with the small nuclei (high power).

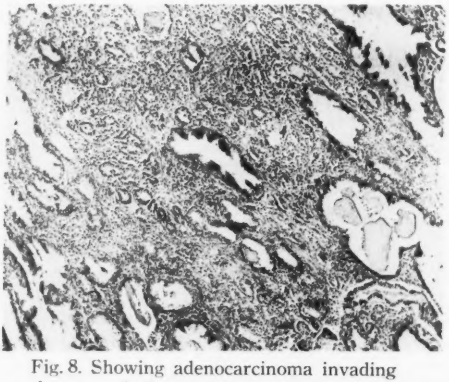

Fig. 8. Showing adenocarcinoma invading the normal tissue of the prostate (low power).

[GANN, Vol. 43, April, 1952]

INVESTIGATIONS ON THE NATURE OF K. I. K. FACTOR IN GASTRIC JUICE FROM GASTRIC CANCER PATIENTS.

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In 1937 Kozawa, Iwatsuru and Kawaguchi[1] found the fact that an eminent decrease of erythrocyte number takes place in rabbit by the intravenous injection of cancerous gastric juice. They named this phenomenon as K. I. K. reaction, and the agent as K. I. K. factor.

We have described the procedure of K. I. K. reaction precisely in our previous paper,[4] which is followed by a second paper on the modified new method, consisting of concentration of the factor by means of precipitation with methanol and of subcutaneous injection in rabbit.

In this report we deal with the chemical nature of K. I. K. factor.

Regarding this factor, the following facts were already demonstrated[2)3)]: 1) The factor does not dialyse through semi-permeable membrane. 2) The factor is not destroyed by heating at 100°C for 5 minutes. 3) The factor is probably destroyed by combustion.

Recently we have persued some other properties of this factor.

Experiments

The full details concerning materials and methods were described in our former report (On a biological method for the diagnosis of gastrict cancer: Gann 42, 50, 1951). The anemiogenic effect of the material used in the experiment was previously proved by injecting intravenously to rabbits

(1) Precipitation with sulfosalicylic acid and metaphosphoric acid.

Half the volume of 25% sulfosalicylic acid (or 10% meta-phosphoric acid) is added to the gastric juice, and then centrifuged.

The precipitate is dissolved in N/10 NaOH, and then neutralized with N/10 HCl—(A). Both this (A) and the supernatant—(B) are each dialyzed against running water for 48 hours, and again dialyzed with distilled water for 12 hours. After this procedure, 2–3 cc of (A) and (B) are injected intravenously to rabbits for 2–3 days successively to test their anemiogenic effect. The results are shown in Table 1.

Table 1.

Case number	K. I. K. reaction		
	Original gastric juice	Precipitate (A)	Supernatant (B)
Sulfosalicylic acid 1	+	++	−
〃 2	++	++	−
Meta-phosphoric acid 1	+	+	−
〃 2	++	++	−

(2) Fractionation with ammonium sulfate.

The gastric juice is brought to 1/2 saturation with ammonium sulfate, and after separation of the precipitate—(C) thus produced, the supernatant is brought to full saturation with ammonium sulfate, then separated to precipitate—(D) and the filtrate—(E).

These 3 fractions are thoroughly dialysed against running water to remove the salt, and then tested as to their anemiogenic effect showing the following results. (Table 2)

Table 2.

Fraction	Case number	K. I. K. reaction
1/2 saturation (C)	1	not tested
	2	++
	3	+
	4	+
Full saturation (D)	1	+
	2	++
	3	+
	4	+
Filtrate (E)	1	−
	2	−
	3	−
	4	−

Original gastric juice	1	++
	2	++
	3	+
	4	++

(3) **Extraction with ether.**

The gastric juice is extracted with ether, then, from the unextractable residue ether is thoroughly removed by warming and ventilation, and tested for its anemiogenic effect. (Table 3)

Table 3.

	Case number	K. I. K. R.
Original gastric juice	1	+
	2	+
	3	++
After extraction	1	++
	2	+
	3	+

(4) **Adsorption with kaoline and its elution.**

1 gm of kaoline is added to 10 cc of the material and acidified with N/10 HCl to pH 6.0. After being kept in ice box for 24 hours, it was centrifuged to separate and remove the supernatant, which proved negative K. I. K. reaction. 10 cc of distilled water is added to the precipitated kaoline, and adjusted to pH 8.0 using N/10 NaOH.

This is again kept in ice box for 24 hours and centrifuged to separate the supernatant, which after dialysis is tested for its anemiogenic effect. The results are shown in Table 4.

In the following experiments, pleural effusion and ascites from malignancy are also used as material, instead of gastric juice. The fact that these fluids also indicate positive K. I. K. reaction was already proved by us.

Table 4.

Case number	Diagnosis	Material used	K. I. K. reaction after adsorption and elution
1	Gastric cancer	Gastric juice	++
2	〃	〃	+

3	〃	〃	+
4	〃	〃	+
5	Endothelioma of the pleura	Pleural effusion	+
6	〃	〃	+
7	Peritonitis carcinomatosa	Ascites	+
8	Tuberculous pleurisy	Pleural effusion	−

(5) Digestion with pancreatine.

To the material, after dialysis, is added 1/10 volume of 5% pancreatine suspension, 1 cc of chloroform and 2 cc of toluene, adjusted to pH 8.1 using N/10 NaOH, sufficiently shaken, and then kept in incubator. After 48 hours, it is centrifuged to separate the supernatant, which is dialyzed for 48 hours and concentrated to 2 cc by means of Faustheim's apparatus. The results using this material are shown in Table 5.

Table 5.

Case number	Diagnosis	Material used	K. I. K. reaction	
			Before digestion	After digestion
1	Gastric cancer	Gastric juice	+	−
2	〃	〃	+	−
3	〃	〃	+	−
4	〃	〃	+	−
5	Endothelioma of the pleura	Pleural effusion	+	−

Summary

The properties of the anemiogenic factor contained in the gastric juice of gastric cancer patient are studied. From the data revealed in these experiments and the facts involved in the method of the K. I. K. reaction, we found the following natures of K. I. K. factor:—1) It is non-dialysable, 2) thermostable, 3) water-soluble but not soluble in ether, 4) precipitated by sulfosalicylic acid, meta-phosphoric acid, and also by ammonium sulfate at 1/2 and full saturations, 5) adsorbed in acid solution on kaoline, and eluted in alkaline solution, and 6) inactivated by digestion with pancreatine.

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3. Iwatsuru, R. et al.: Gann 32 (1938) (Japanese.)
4. Iwatsuru, R., and Kato, I.: Gann 42 (1951)

要　　旨

K. I. K. 因子の性状について

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我々は，胃癌患者胃液中のK. I. K. 因子の性状に関して，次の事実を認めた。即ち，この因子は，1. 透析されない。2. 耐熱性である。3. 水溶性，エーテルに不溶。4. スルフオサリチル酸等の蛋白沈澱剤によって沈澱し，また，硫酸アンモンの半飽和によるも，完全飽和によるも沈澱する。5. 酸性溶液においてカオリンに吸着され，アルカリ性にすれば離される。6. パンクレアチンで消化すると，作用が失われる。（文部省科学研究費による）

[GANN, Vol. 43, April, 1952]

TOXOHORMONE AND THYMUS INVOLUTION IN TUMOR BEARING ANIMALS. A FOURTH STUDY ON TOXOHORMONE, A CHARACTERISTIC TOXIC SUBSTANCE PRODUCED BY CANCER TISSUE

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INTRODUCTION

In animals bearing malignant tumors there are several systemic changes sufficiently well marked to serve as objects of experimental study. Of these changes the depression of catalase, especially liver catalase, and reduced concentration of hemoglobin associate themselves together in one group, since both catalase and hemoglobin are iron-protein compounds. Our recent findings showing that toxohormone interferes in some way with the utilization of iron for the synthesis of catalase (1) may be said to have rendered self-evident that this characteristic toxic substance produced by cancer tissue may be the cause of this group of the systemic changes.

Another group of systemic changes in cancer much discussed lately consists of the adrenal enlargement and thymus involution (2–6). The enlargement of the adrenals is said to be accompanied by a marked reduction in their ascorbic acid and lipid contents, and is interpreted as representing a reduced cortical activity, and this, together with the constant involution of thymus, is being considered in connection with the so-called adaptation syndrome in stress reaction.

In recent experiments we made some attempts to correlate the adrenal enlargement and thymus atrophy in tumor bearing animals with the activity of toxohormone. Could these organ changes in neoplastic disease also be attributed to the action of toxohormone, which we now know may be responsible for the first group of the systemic changes referred to above?

The results of these experiments led us to the conclusion that adrenal enlargement and thymus involution observed in tumor bearing animals may be more or less unrelated and independent phenomena, since we found that a single injection of toxohormone induces a striking involution of the thymus without affecting the adrenal weight. In this paper we propose to present our experimental data and to discuss the significance of the findings.

Material and Methods

Young adults, weighing 15–20 g, of a mixed strain albino mice were used throughout the present experiments. The tumor used was NF strain of our transplantable mouse sarcoma previously described (1). It is a fibrosarcoma of spontaneous origin, maintained by subcutaneous transplantation now over 60 generations in this laboratory. It grows with rapidity and generally produces tumors of 4~5 g weight in 3~4 weeks. Mice were fed on the diet of mixed grains with occasional supply of dried fish and green vegetables.

Organs were carefully dissected out and were weighed as soon as practicable after killing the animals, using a torsion balance. Since right and left adrenals were often much different in their weights, their combined weights were taken for tabulation. The organ weights were recalculated to per 100 g of the body weight (carcass weight in the case of tumor bearing mice).

Thymus and Adrenal Weights in Normal and Tumor Bearing Mice

Our data on thymus and adrenal weights (mg) in normal and tumor bearing mice may be tabulated as follows:

Normal Mouse No.		Adrenals	Thymus
1	♂	25.9	276.0
2	〃	27.5	191.0
3	〃	23.1	128.0
4	〃	32.3	121.0
5	〃	17.9	94.9
6	〃	14.1	75.1
7	〃	27.6	72.0
8	〃	17.9	65.4
9	〃	17.4	58.4
10	〃	24.1	52.0
Average for males		22.7	113.3
11	♀	31.0	227.0
12	〃	40.6	217.0
13	〃	33.3	211.0
14	〃	25.4	200.0
15	〃	28.1	190.0
16	〃	24.5	169.0
17	〃	27.6	160.4
18	〃	24.0	159.0
19	〃	25.5	129.0
20	〃	32.8	129.0

21	♀	30.0	103.0
22	〃	34.1	98.2
23	〃	36.4	87.0
24	〃	27.5	84.0
25	〃	39.2	45.2
Average for females		30.6	147.2

Tumor	Mouse No.	Absolute wt. of Tumor (g)	Adrenals	Thymus
1	♂	5.0	22.3	81.2
2	〃	5.0	20.3	51.9
3	〃	0.7	55.4	46.8
4	〃	7.0	20.0	41.2
5	〃	1.0	20.9	31.0
6	〃	2.5	25.1	30.6
7	〃	4.0	20.0	29.2
8	〃	2.3	32.1	20.2
Average for males		3.4	27.0	41.5
9	♀	1.5	28.1	148.0
10	〃	5.2	42.8	105.0
11	〃	2.7	72.0	69.0
12	〃	2.8	23.7	68.0
13	〃	3.5	16.4	54.0
14	〃	3.7	30.7	51.0
15	〃	3.0	28.7	49.3
16	〃	5.0	19.3	47.5
17	〃	5.0	38.5	42.2
18	〃	5.0	30.6	40.0
19	〃	5.7	41.9	39.9
20	〃	5.6	40.4	39.5
21	〃	1.7	24.0	37.6
22	〃	3.6	18.5	35.2
23	〃	5.9	25.1	34.6
24	〃	7.5	24.2	32.7
25	〃	2.4	20.2	32.3
26	〃	4.5	56.2	30.5
27	〃	2.4	31.0	29.1
28	〃	4.0	36.8	25.4
Average for females		4.0	32.4	50.0

An analysis of the above data permits the following statements;—

1. The adrenals tend to be larger in the female than in the male, and in tumor bearing than in non-tumor bearing mice. The differences, however, are

disappointingly slight.

2. The thymus weight shows no material difference as to sexes, but the difference between normal and tumor bearing mice is altogether very striking. In tumor bearing mice the thymus was generally less than the average normal size, and in some 60 percent of the cases the organ was smaller than the smallest that was found among normal mice.

3. The degree of the thymus involution may be related to the size of the tumor, but this relation was not always apparent.

4. The degree of the thymus involution is plainly not correlated to the adrenal enlargement.

Effect of Injections of Toxohormone on Thymus and Adrenal Weights.

Having verified the occurrence of a marked thymus involution in mice bearing a transplanted sarcoma, we next tested the possibility of reproducing the similar thymus change in normal mice by the injection of isolated toxohormone fraction.

The toxohormone fraction was prepared from our transplantable sarcoma NF, according to the copper sulphate precipitation method we previously described (7), namely, by extracting dried tumor tissue with distilled water under heating, precipitating the active material from the clear watery solution with alcohol, and by re-precipitating with copper sulphate, followed by the removal of copper and impurities with hydrochloric acid. The final preparation was washed with ether. This method yields from this type of tumor a fraction which is capable of markedly depressing liver catalase of normal mice in 10 mg doses (1).

Normal mice were injected intraperitoneally with 10 or 20 mg of this fraction, and thymus and adrenal weights were determined 24 hours, 48 hours and 3 days after the injection. The following results were obtained:—

Amount of Toxohormone injected	Time after injection	Mouse No.	Adrenals	Thymus
10 mg	48 hrs.	1	32.5	72.0
	3 days	2	24.6	87.0
		3	25.7	107.8
20 mg	24 hrs.	4	21.4	51.4
		5	37.5	28.1
		6	22.3	48.0
		7	40.7	45.7
	48 hrs.	8	23.0	39.5
		9	23.8	31.7

		10	26.0	30.7
		11	16.2	23.8
		12	16.9	22.8
	3 days	13	28.3	29.2

The above results demonstrate that a marked involution of thymus was induced in normal mice 48 hours after a single injection of 20 mg of the toxohormone fraction. 10 mg was inadequate, and 24 hours after injection was too early to show the effect. This would mean that a larger amount and longer time are required to produce thymus involution than to depress the liver catalase, since with the fraction used the liver catalase can be greatly reduced 24 hours after the injection of 10 mg. The absence of adrenal weight increase was quite as clear at the positive effect on the thymus.

Control Experiments: For control to the above experiments we tested the effect of some other fractions from the same tumor tissue which are known to be without the toxohormone activity. These included: 1, Nucleo-histone fraction obtained by precipitation from fresh tumor extract with calcium chloride; 2, nucleoprotein fraction precipitated with acetic acid from the same extract after the removal of the nucleohistone fraction; and 3, peptone fraction, which is the filtrate after the removal of the crude alcoholic precipitate in our original method of preparing toxohormone.

In addition to the tumor fractions, a mixture of normal mouse tissues (liver, kidney, lung, spleen, lymphnodes, stomach, small intestine, etc.) was treated according to the method for isolating the toxohormone fraction from tumor tissue, and the copper sulphate precipitate obtained was tested as normal tissue control.

All these materials were injected in 20 mg amounts.

None of these control materials produced thymus involution, or changes in adrenal weight, as may be apparent in the following figures:—

Material injected	Time after injection	Mouse No.	Adrenals	Thymus
Tumor necleohistone	24 hrs.	1	30.4	70.0
	48 hrs.	2	31.1	93.3
		3	23.4	49.6
	3 days	4	18.6	171.0
		5	19.5	145.0
Tumor nucleoprotein	24 hrs.	6	38.4	55.3
	48 hrs.	7	27.1	87.6
		8	22.1	87.0

	3 days	9	33.1	112.0
		10	24.3	79.0
	24 hrs.	11	29.4	194.0
	48 hrs.	12	13.1	207.0
Tumor peptone		13	23.9	132.0
	3 days	14	25.0	112.0
		15	23.3	46.3
	24 hrs.	16	39.4	75.2
		17	19.2	64.1
	48 hrs.	18	18.2	121.0
Normal tissue fraction		19	21.9	69.8
		20	24.4	45.2
	3 days	21	15.9	148.0
		22	27.6	71.8

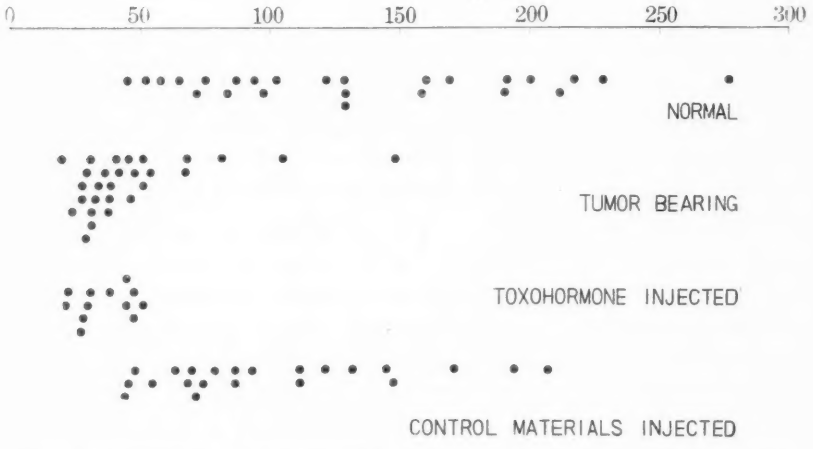

Chart 1. Thymus weight (mg per 100 g body weight) in various experimental groups of mice. Toxohormone group includes only those receiving 20 mg. doses.

Discussion

In the observations described in this paper the marked involution of the thymus in tumor bearing mice and in normal mice injected with an adequate amount of toxohormone fraction stands out with exceptional clearness. Compared with the thymus involution, adrenal enlargement in tumor bearing mice was so slight as to be practically negligible, and there was no perceptible increase in adrenal weight in normal mice injected with toxohormone.

It seems clear that the thymus involution occurring in tumor bearing animals is ascribable to the action of a substance contained in the toxohormone fraction. Whether or not this active substance is identical with that which causes the depression of liver catalase cannot now be determined, and it can only be said that our toxohormone concentrate carries both actions, which are not shown by other fractions isolated from the same tumor tissue or by the "toxohormone fraction" from normal tissues.

The involution of the thymus accompanied by the adrenal enlargement was discussed by some authors in the light of the adaptation syndrome of the so-called stress reaction (2, 5), but the fact that a marked thymus involution may take place under the influence of toxohormone, without the corresponding adrenal hypertrophy, seems to render the adaptation syndrome theory inapplicable to the case under consideration. It would seem much more likely that the thymus involution may be connected with the disturbance of nitrogen metabolism, and it may be looked upon as an early sign of the wasting of body protein in tumor animals. The known conditions, such as malnutrition, infection, intoxication, etc., under which the thymus atrophy ordinarily occurs support this view. It is conceivable that toxohormone, through its depressing action on the liver cell function, may adversely affect protein synthesis, and Yeakel (8) suggested that the increased size of the liver of the tumor bearing animals is related to the increased anabolism of proteins induced by the tumor. These ideas bring us back to our early suggestion that toxohormone may play an important role in producing the so-called cancer cachexia (9, 10).

Conclusions

The marked involution of the thymus, such as very frequently occurs in tumor bearing animals, can be induced in normal mice by a single injection of a tumor fraction which carries toxohormone, the characteristic toxic substance produced by cancer tissue which has the action of depressing the liver catalase. Other tumor fractions devoid of the toxohormone activity or a fraction similar to the toxohormone fraction isolated from mixed normal tissues, failed to affect the thymus.

The thymus involution in these cases can hardly be regarded as a part of the so-called adaptation syndrome, since the change is not accompnied by the adrenal enlargement, which is the integral part of the syndrome. We consider it most probable that the phenomenon may be based on adverse protein metabolism, and may be an early sign of the wasting of body protein, which occurs in neoplastic disease and which ultimately leads to the condition known as cancer cachexia.

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要　　旨

トキソホルモンと擔癌動物の胸腺退縮

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(癌研究所・科学研究所)

担癌マウス及びラッテにおいて胸腺の顯著な退縮が見られるが，それと同程度の変化がトキソホルモン濃縮物の適量の一回注射で正常マウスに起ることを見付けた。

担癌動物の胸腺退縮は副腎の肥大を伴っている点から，stress に対する適應反應と関係をつけようとしている学者もあるが，我々の使用したマウス肉腫では，胸腺退縮の著しいにかかわらず，副腎の肥大はすこぶる微弱で問題にし難い。殊にトキソホルモン注射により胸腺は著明に退縮するのに，副腎は殆んど変らない点から見て，この兩者は密接な相関々係にあるものとは考えられない。

胸腺の退縮は一般に動物体の種々な不良な状態と関連していて，トキソホルモンによる場合も恐らく体蛋白の消耗を示すものと思われる。トキソホルモンによって起る肝細胞機能の障害が，不利な蛋白代謝を招き，終局において惡液質の状態まで進行することは推測に難くない。胸腺の退縮はその第一歩を表徴すると解釈出來る。（文部省科学研究費による）